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(54) Title: IMMUNE MEDIATORS AND RELATED METHODS

(57) Abstract: The present invention relates to nucleic acids encoding single chain MHC class II molecules that form multimers via inter-chain multimerization domains, and methods of treating autoimmune disease using the same.



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## IMMUNE MEDIATORS AND RELATED METHODS

### CROSS-REFERENCES TO RELATED APPLICATIONS

This application is related to U.S. Serial No. 60/191,274, filed March 22, 2000; U.S. Serial No. 60/204,249, filed May 15, 2000; and U.S. Serial No. 60/264,003, filed January 23, 2001. Each of the aforementioned applications are herein incorporated by reference in their entirety.

This application is also related to U.S. Serial No. 09/261,811, filed March 3, 1999; which is a continuation of U.S. Serial No. 08/657,581, filed June 7, 1996, now abandoned; which is a continuation in part of U.S. Serial No. 08/480,002, filed June 7, 1995, now abandoned; U.S. Serial No 09/184,692, filed November 2, 1998, now abandoned; U.S. Serial No. 08/483,241, filed June 7, 1995; U.S. Serial No. 08/482,133, filed June 7, 1995; and U.S. Provisional Application No. 60/005,964, filed October 27, 1995.

### BACKGROUND OF THE INVENTION

T cells, unlike B cells, do not directly recognize antigens. Instead, an accessory cell must first process an antigen and present it in association with an MHC molecule in order to elicit a T cell-mediated immunological response. The major function of MHC glycoproteins appears to be the binding and presentation of processed antigen in the form of short antigenic peptides.

In addition to binding foreign or "non-self antigenic peptides," MHC molecules can also bind "self" peptides. If T lymphocytes then respond to cells presenting "self" or autoantigenic peptides, a condition of autoimmunity results. Over 30 autoimmune diseases are presently known, including myasthenia gravis (MG), multiple sclerosis (MS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), insulin-dependent diabetes mellitus (IDDM), etc. Characteristic of these diseases is an attack by the immune system on the tissues of the host. In non-diseased individuals, such attack does not occur because the immune system recognizes these tissues as "self." Autoimmunity occurs when a specific adaptive immune response is mounted against self tissue antigens.

There is therefore currently a great interest in developing pharmaceuticals based on the growing understanding of the structure and function of the major

histocompatibility complex (MHC) antigens. Identification of synthetic autoantigenic peptides, and demonstration that these peptides bind selectively to MHC molecules associated with disease and that stimulates T cells would help to implicate a particular peptide or peptide:MHC complex in susceptibility to an autoimmune disease. In

5 particular, the development of single chain MHC class II complexes would be particularly useful in treatment of a number of diseases associated with antigen presentation by MHC molecules. Furthermore, the development of single chain, multimeric complexes would be of interest (*see, e.g.*, WO 93/10220, WO 98/05684, WO 97/35991, WO 98/03552, WO 99/13095, WO 98/06749, WO 99/09064, and U.S. Patent 5,869,270).

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### SUMMARY OF THE INVENTION

The present invention provides recombinant nucleic acid constructs that encode single chain, recombinant MHC class II molecules comprising a  $\beta 1$  domain and an  $\alpha 1$  domain that may or may not be further linked to an antigenic peptide. In one

15 embodiment, the single chain polypeptide is a  $\beta 1$  domain and an  $\alpha 1$  domain. In another embodiment, the single chain polypeptide is a  $\beta 1$  domain- $\beta 2$  domain (a  $\beta$  chain) and an  $\alpha 1$  domain- $\alpha 2$  domain (an  $\alpha$  chain). The single chain constructs of the invention can be further dimerized or multimerized by inter-chain fusion. The fusion sequence (also referred to as a dimerization or multimerization sequence) can be any sequence that

20 allows for covalent or non-covalent linkages between the molecules of the invention. As shown below, a preferred means for carrying this out is through use of segments from immunoglobulin family proteins (e.g., antibodies, MHC molecules, T cell receptors and the like) that have cysteine residues capable of forming interchain disulfide bonds (e.g., constant regions from Ig light chains, e.g. C $\kappa$  or C $\lambda$ , or constant regions from Ig heavy

25 chains, e.g., CH1, hinge, CH2, or CH3). In another embodiment, a leucine zipper domain forms a non-covalent linkage. One of skill will recognize that any of a number of polypeptide sequences can be used for this purpose. The single chain molecules of the invention thus can be multimers wherein each single chain molecule is from a different MHC class II allele. In addition, each single chain molecule in the multimer can be

30 bound to a different antigen.

In one embodiment, monomeric and dimeric forms of recombinant single chain mouse I-AS-peptide complexes, fused to an antigenic MBP 90-101 peptide with

flexible linkers were constructed. The recombinant single chain I-AS proteins share structural similarity to that of crystallized native human MHC class II protein as determined by protein modeling. The recombinant single chain proteins were expressed in *E. coli* and in an insect expression system and purified by affinity chromatography and FPLC. The purified single chain recombinant I-AS proteins showed *in vitro* biological activity as assayed using an antigen-specific mouse T cell clone. The *in vivo* activity of the recombinant single chain I-AS fusion proteins in the EAE model using susceptible SJL mice shows that treatment with the recombinant single chain I-AS proteins prevents mortality and significantly reduces paralysis induced by myelin homogenate.

Histological examination of sections from animal spinal cord reveals that these treatments also reduce the inflammatory lesions. These results demonstrate that the single chain MHC class II molecules have therapeutic benefit as antigen-specific drugs for the treatment of autoimmune diseases.

In another embodiment, novel linkers are provided for forming single chain MHC class II molecules. These linkers can be used with the multimer constructs described above. In another embodiment, the constructs of the invention are optimized for prokaryotic expression, using codons adjusted for *E. coli* codon bias.

The present invention also provides MHC class II heterodimers, wherein a recombinant  $\beta$  chain and a recombinant  $\alpha$  chain are covalently linked using polypeptide fusion segments, e.g., from immunoglobulin family proteins (e.g., antibodies, MHC molecules, T cell receptors and the like) that have cysteine residues capable of forming interchain disulfide bonds (e.g., constant regions from Ig light chains, e.g. C $\kappa$  or C $\lambda$ , or constant regions from Ig heavy chains, e.g., CH1, hinge, CH2, or CH3). Such a heterodimer can also be "dimerized" or "multimerized" by the use of additional fusion domains, such as leucine zipper domains or immunoglobulin domains (see Figure 10).

### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Schematic structure of the recombinant single chain I-AS .MBP. $\beta$ 1 $\alpha$ 1 (monomer) and I-AS.MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.CK (dimer) proteins.

Figure 2. *In vitro* biological activities of the recombinant I-AS proteins compared with APC+ antigenic peptide in the mouse T cell clone, HS-1.

A. I-AS.MBP. $\beta$ 1 $\alpha$ 1, monomer, B. I-AS.MBP.Ck, dimer, and C. APC+ antigenic

peptide. The positive (anti-CD3) and the negative (HS-1 cell alone) controls are also shown in each panel.

Figure 3. Diagram of the EAE model and standard for EAE scoring.

Figure 4. The EAE model studies. The recombinant I-AS proteins were administered to SJL mice on day 1, 4, 7, and II by i.v. injection after inducing the disease with myelin emulsified in CFA. The animals were evaluated for neurological dysfunction. Panel A: Untreated, injected with same amount of PBS solution. Panel B: Treated with the recombinant I-AS.MBP.Ck protein, a dimer form. Panel C: Treated with the recombinant I-AS. $\beta$ 1 $\alpha$ 1. This recombinant molecule does not carry the antigenic peptide. Panel D: Treated with the recombinant I-AS.MBP. $\beta$ 1 $\alpha$ 1, a monomer form.

Figure 5 shows a schematic representation of a  $\beta$ 1- $\alpha$ 1 single chain MHC class II peptide complex that is a dimer with two peptide specificities.

Figure 6 shows a schematic representation of a  $\beta$ 1 $\beta$ 2- $\alpha$ 1 $\alpha$ 2 single chain MHC class II peptide complex that is a dimer with two peptide specificities.

Figure 7 shows a schematic representation of a  $\beta$ 1 $\beta$ 2- $\alpha$ 1 $\alpha$ 2 single chain MHC class II peptide complex that is a tetramer with four peptide specificities.

Figure 8 shows a schematic representation of a  $\beta$ 1 $\beta$ 2- $\alpha$ 1 $\alpha$ 2 single chain MHC class II peptide complex that is a tetramer with two peptide specificities and two different MHC class II alleles.

Figure 9 shows the effect of different recombinant MHC class II molecules on the development of EAD (day 60+).

Figure 10. Diagram of  $\beta$ 1- $\alpha$ 1 single chain MHC class II peptide complex; diagram of recombinant  $\beta$ 1 $\beta$ 2 chains fused to recombinant  $\alpha$ 1 $\alpha$ 2 chains via a fusion domain from an immunoglobulin; and diagram of multimerized MHC class II molecules.

Figure 11. Sequence comparison of mouse CO608 single chain molecules.

Figure 12. Sequence comparison of mouse CO561 single chain molecules.

## DETAILED DESCRIPTION OF THE INVENTION

### Introduction

The present invention provides recombinant DNA constructs that encode single chain MHC class II molecules that may or may not be further linked to an antigenic peptide. Typically, the constructs comprise a first DNA segment encoding a  $\beta$ 1 domain

of a selected MHC class II molecule; a second DNA segment encoding a  $\alpha 1$  domain of the selected MHC class II molecule; and a first linker DNA segment connecting in-frame the first and second DNA segments; wherein linkage of the first DNA segment to the second DNA segment by the first linker DNA segment results in a fused first DNA-first linker-second DNA polysegment. The constructs of the invention may also comprise a third DNA segment encoding an antigenic peptide capable of associating with a peptide binding groove of the selected MHC class II molecule and a second linker DNA segment connecting in-frame the third DNA segment to the fused first DNA-first linker-second DNA polysegment.

10 In another embodiment, the present invention also provides recombinant components of an MHC class II heterodimer, which comprise a fusion domain. One recombinant component comprises a  $\beta 1$  domain, or optionally a  $\beta 1$  domain- $\beta 2$  domain (i.e., a  $\beta$  chain). One recombinant component comprises an  $\alpha 1$  domain, or optionally an  $\alpha 1$  domain- $\alpha 2$  domain (i.e., an  $\alpha$  chain). The two recombinant chains are linked, either covalently, e.g., via a disulfide bond, or non covalently, using the fusion domain. Such molecules can also be made into multimers using additional fusion or multimerization domains. In one embodiment, the invention provides the following recombinant components of an MHC class II heterodimer: pCB220, which is an IAS.MBP.alpha chain fused to an IgG2a CH1 and truncated hinge region; pCB223, which is an IAS.MBP.alpha chain fused to an IgG2a CH1.H.CH2.CH3; and pCB229, which is an IAS.MBP.beta chain fused to a mouse C $\kappa$  domain. These recombinant components can be fused via the fusion domain to form a MHC class II heterodimer molecule that is covalently linked via a disulfide bond at the fusion domain.

25 The present invention provides single chain MHC class II molecules that comprise an additional polypeptide sequence that allows for inter-chain dimerization of the single chain molecules of the invention. The additional polypeptide allows multimerization of the single chain MHC class II molecules, to produce, e.g., dimers and tetramers. The sequence can be any sequence that allows for covalent or non-covalent linkages between the molecules of the invention. In one embodiment, the single chain molecules are covalently linked using chemical methods known to those of skill in the art, e.g., photoaffinity methods or homo-bifunctional protein cross-linkers (*see, e.g.,* Hermanson *et al., Bioconjugate Techniques*, (1996)). In one embodiment, the molecules

are covalently linked using heterobifunctional protein cross-linkers. As shown below, one means for carrying this out is through use of segments from immunoglobulin family proteins (e.g., antibodies, MHC molecules, T cell receptors and the like) that have cysteine residues capable of forming interchain disulfide bonds. An example shown below is the use of the constant region of the kappa chain of an antibody (C $\kappa$ ), from either a heavy or a light chain. Other dimerization sequences include a leucine zipper, a STAT protein N-terminal domain, or the FK506 binding protein (*see, e.g., O'Shea, Science* 254: 539 (1991), Barahmand-Pour *et al., Curr. Top. Microbiol. Immunol.* 211:121-128 (1996); Klemm *et al., Annu. Rev. Immunol.* 16:569-592 (1998); Ho *et al., Nature* 382:822-826 (1996)). One of skill will recognize that any of a number of polypeptide sequences can be used for this purpose.

In addition, the multimeric, single chain class II molecules of the invention comprise at least two different MHC class II alleles that are associated with an autoimmune disease state, and/or at least two different autoantigenic peptides that are associated with a particular autoimmune disease state. In one example, the multimeric, single chain class II molecules have chains from different DR2 alleles, e.g., DRB5\*0101 and DRB1\*1501. In another embodiment, the autoantigenic peptides are peptides associated with multiple sclerosis, e.g., MBP (e.g., amino acid residues 83-102Y83), PLP (e.g., amino acid residues 40-60, 89-106, 95-117, and 185-206); and MOG. In addition, other antigens associated with autoimmune disease, such as acetylcholine receptor and type II collagen, can be linked to the single chain molecules of the invention..

In a further embodiment, the single chain class II molecules of the invention have novel linkers, as described herein. The mammalian MHC class II single chain constructs of the invention may also be constructed to use preferred prokaryotic codons, for expression, e.g., in *E. coli*, using codon preference tables and methods known to those of skill in the art.

## Definitions

Prior to setting forth the invention, it may be helpful to an understanding thereof to provide definitions of certain terms to be used hereinafter:

Single chain MHC class II molecule: As used herein this term refers to a fusion protein such as the recombinant single chain MHC class II complex of the

invention, which optionally also is fused to a peptide to form a single chain MHC class:peptide complex. The fusion proteins of the invention can also be multimers, having two, four or more single chain molecules linked covalently or non-covalently through multimerization domains in the single chain molecule. A single chain molecule of the invention typically comprises at least an MHC class II  $\beta 1$  domain and an MHC class II  $\alpha 1$  domain, optionally  $\beta 1$   $\beta 2$   $\alpha 1$   $\alpha 2$  domains or any combination thereof in any order. Such molecules are also known as "fused heterodimers." Optionally, the single chain molecules are soluble, that is, they lack the naturally occurring cytoplasmic and transmembrane MHC class II domains.

10                    A domain of a selected MHC molecule: A portion of an MHC domain which is sufficient to form, either alone, or in combination with another portion of an MHC domain, a peptide binding site which is capable of presenting an antigenic peptide in such a fashion that it is recognized by a T cell receptor. Such MHC domains would include the extracellular portion of the two polypeptide chains of Class II MHC. This would include the  $\alpha$  chain ( $\alpha 1$  and  $\alpha 2$  domains) and  $\beta$  chain ( $\beta 1$  and  $\beta 2$  domains) of Class II MHC. This would include  $\beta 1$  and  $\alpha 1$ ,  $\beta 1$ ,  $\beta 2$  and  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 1$  or  $\alpha 2$  independent of the other, or  $\alpha 1$  and  $\alpha 2$  in tandem ( $\alpha 1\alpha 2$ ). It would also include  $\beta 1$  or  $\beta 2$  independent of the other, or  $\beta 1$  and  $\beta 2$  in tandem ( $\beta 1\beta 2$ ). This would also include any suitable combination of the  $\alpha 1$ ,  $\alpha 2$ ,  $\beta 1$ , and  $\beta 2$  domains. The domains can be directly linked, or can be linked via an amino acid linker.

20                    Linker DNA segment: A segment of DNA encoding from about 1 to about 50, preferably from about 5 to about 25 amino acids, which forms a flexible link between two DNA segments. This flexible link allows the two DNA segments to attain a proper configuration, such as an MHC peptide binding groove, or allows a peptide to properly bind into such a groove.

25                    Antigenic peptide: The immunological properties of MHC histocompatibility proteins are largely defined by the antigenic peptide that is bound to them. An antigenic peptide is one which contains an epitope (an amino acid sequence) recognized by immune cells, e.g., T cells, and is capable of stimulating an MHC-mediated immune response.. Antigenic peptides for a number of autoimmune diseases are known. For example, in experimentally induced autoimmune diseases, antigens involved in pathogenesis have been characterized: in arthritis in rat and mouse, native type II collagen



is identified in collagen-induced arthritis, and mycobacterial heat shock protein in adjuvant arthritis (Stuart *et al.*, *Ann. Rev. Immunol.* 2:199-218, 1984; and van Eden *et al.*, *Nature* 331:171-173, 1988); thyroglobulin has been identified in experimental allergic thyroiditis (EAT) in mice (Marion *et al.*, *J. Exp. Med.* 152:1115-1120, 1988); acetylcholine receptor (AChR) in experimental allergic myasthenia gravis (EAMG) (Lindstrom *et al.*, *Adv. Immunol.* 42:233-284, 1988) ; and myelin basic protein (MBP) and proteolipid protein (PLP) in experimental allergic encephalomyelitis (EAE) in mouse and rat (Acha-Orbea *et al.*, *Ann. Rev. Imm.* 7:377-405, 1989). In addition, target antigens have been identified in humans: type II collagen in human rheumatoid arthritis (Holoshitz *et al.*, *Lancet* ii:305-309, 1986), acetylcholine receptor in myasthenia gravis (Lindstrom *et al.*, *Adv. Immunol.* 42:233-284, 1988), and MBP, PLP, and MOG in multiple sclerosis in humans.

MHC: The major histocompatibility complex (MHC) is a family of highly polymorphic proteins, divided into two classes, Class I and Class II, which are membrane-associated and present antigen to T lymphocytes (T cells). MHC Class I and Class II molecules are distinguished by the types of cells on which they are expressed, and by the subsets of T cells which recognize them. Class I MHC molecules (e.g., HLA-A, -B and -C molecules in the human system) are expressed on almost all nucleated cells and are recognized by cytotoxic T lymphocytes (CTL), which then destroy the antigen-bearing cells. Class II MHC molecules (HLA-DP, -DQ and -DR, for example, in humans) are expressed primarily on the surface of antigen-presenting cells, such as B lymphocytes, dendritic cells, macrophages, and the like. Class II MHC is recognized by CD4<sup>+</sup> T helper lymphocytes (T<sub>H</sub>). T<sub>H</sub> cells induce proliferation of both B and T lymphocytes, thus amplifying the immune response to the particular antigenic peptide that is displayed (Takahashi, *Microbiol. Immunol.*, 37:1-9, 1993).

Two distinct antigen processing pathways are associated with the two MHC classes. Intracellular antigens, synthesized inside of the cell, such as from viral or newly synthesized cellular proteins, for example, are processed and presented by Class I MHC. Exogenous antigens, taken up by the antigen-presenting cell (APC) from outside of the cell through endocytosis, are processed and presented by Class II MHC. After the antigenic material is proteolytically processed by the MHC-bearing cell, the resulting antigenic peptide forms a complex with the antigen binding groove of the MHC molecule

through various noncovalent associations. The MHC-peptide complex on the cell surface is recognized by a specific T cell receptor on a cytotoxic or helper T cell.

The MHC of humans (also referred to as human leukocyte antigens (HLA)) on chromosome 6 has three loci, HLA-A, HLA-B and HLA-C, the first two of which have a large number of alleles encoding alloantigens. An adjacent region, known as HLA-D, is subdivided into HLA-DR, HLA-DQ and HLA-DP. The HLA region is now known as the human MHC region, and is equivalent to the H-2 region in mice. HLA-A, -B and -C resemble mouse H-2K, -D, and -L and are the Class I MHC molecules. HLA-DP, -DQ and -DR resemble mouse I-A and I-E and are the Class II molecules. MHC glycoproteins of both classes have been isolated and characterized (*see Fundamental Immunology*, 2d Ed., Paul (ed.), (1989); and Roitt *et al.*, *Immunology*, 2d Ed., (1989), which are both incorporated herein by reference).

Human MHC Class I molecules consist of a polymorphic type I integral membrane glycoprotein heavy chain of about 46 kD, noncovalently associated with a 12 kD soluble subunit,  $\beta$ 2-microglobulin. The heavy chain consists of two distinct extracellular regions, the membrane distal, peptide binding region formed by the  $\alpha$ 1 and  $\alpha$ 2 domains, and the membrane proximal, CD8-binding region derived from the  $\alpha$ 3 domain.  $\beta$ 2- microglobulin is a single, compact immunoglobulin-like domain that lacks a membrane anchor, and exists either associated with the class I heavy chain or free in plasma (Germain and Margulies, *Annu. Rev. Immunol.* 11:403-50, 1993).

Human MHC Class II is a heterodimeric integral membrane protein. Each dimer consists of one  $\alpha$  and one  $\beta$  chain in noncovalent association. The two chains are similar to each other, with the  $\alpha$  chain having a molecular weight of 32-34 kD and the  $\beta$  chain having a molecular weight of 29-32 kD. Both polypeptide chains contain N-linked oligosaccharide groups and have extracellular amino termini and intracellular carboxy termini.

The extracellular portions of the  $\alpha$  and  $\beta$  chain that comprise the class II molecule have been subdivided into two domains of about 90 amino acids each, called  $\alpha$ 1,  $\alpha$ 2, and  $\beta$ 1,  $\beta$ 2, respectively. The  $\alpha$ 2 and  $\beta$ 2 domains each contain a disulfide-linked loop. The peptide-binding region of the class II molecule is formed by the interaction of the  $\alpha$ 1 and  $\beta$ 1 domains. This interaction results in an open-ended, antigenic peptide-binding groove made up of two  $\alpha$  helices, and an eight-stranded  $\beta$ -pleated sheet platform.

The  $\alpha$  and  $\beta$  chains of Class II molecules are encoded by different MHC genes and are polymorphic (*see Addas et al., Cellular and Molecular Immunology*, 2d Ed. (1994), which is incorporated by reference in its entirety). Within the present invention, a preferred  $\alpha$  chain is DRA\*0101 and a preferred  $\beta$  chain is DR $\beta$ 1\*1501.

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### MHC Class II alleles

The single chain MHC class II:peptide complexes of the present invention can incorporate cDNA from any allele that predisposes or increases the likelihood of susceptibility to a specific autoimmune disease. Specific autoimmune diseases are correlated with specific MHC types. Specific haplotypes have been associated with many of the autoimmune diseases. For example, HLA-DR2<sup>+</sup> and HLA-DR3<sup>+</sup> individuals are at a higher risk than the general population to develop systemic lupus erythematosus (SLE) (Reinertsen *et al.*, *N. Engl. J. Med.* 299:515-18, 1970). Myasthenia gravis has been linked to HLA-D (Safwenberg *et al.*, *Tissue Antigens* 12:136-42, 1978. Susceptibility to rheumatoid arthritis is associated with HLA-D/DR in humans. Methods for identifying which alleles, and subsequently which MHC-encoded polypeptides, are associated with an autoimmune disease are known in the art. Exemplary alleles for IDDM include DR4, DQ8, DR3, DQ3.2.

### 20 Uses of single chain MHC class II molecules

Single chain MHC class II molecules and/or single chain MHC class II:peptide complexes of the present invention can be used as antagonists to therapeutically block the binding of particular T cells and antigen-presenting cells. In addition, the molecules can induce anergy, or proliferative nonresponsiveness, and possibly apoptosis, in targeted T cells, both *in vivo* and *in vitro*. A single chain MHC class II:peptide molecule directed toward a desired autoimmune disease contains the antigenic peptide implicated for that autoimmune disease properly positioned in the binding groove of the MHC molecule, without need for solubilization of MHC or exogenous loading of an independently manufactured peptide.

Previous methods for producing desirable MHC Class II histocompatibility proteins have provided material that contains a mixture of antigenic peptides (Buus *et al.*, *Science* 242:1045-1047, 1988; and Rudensky *et al.*, *Nature* 353:622-627, 1991), which

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can be only partially loaded with a defined antigenic peptide (Watts and McConnel, *Pro. Natl. Acad. Sci. USA* 83:9660-64, 1986; and Ceppellini *et al.*, *Nature* 339:392-94, 1989). Various methods have been developed to produce heterodimers that do not present endogenous antigens (Stern and Wiley, *Cell* 68:465-77, 1992; Ljunggren *et al.*, *Nature* 346:476-80, 1990; and Schumacher *et al.*, *Cell* 62:563-67, 1990) that can be loaded with a peptide of choice. WO 95/23814 and Kozono *et al.* have described production of soluble murine Class II molecules, I-E<sup>dk</sup> and I-A<sup>d</sup>, each with a peptide attached by a linker to the N terminus of the  $\beta$  chain. Ignatowicz *et al.* (*J. Immunol.* 154:38-62, 1995) have expressed membrane-bound I-A<sup>d</sup> with peptide attached. These methods incorporate the use of both membrane-bound heterodimer and soluble heterodimer.

The current invention offers the advantage of a recombinant single chain MHC class II molecule made up of two or more MHC domains joined together via a flexible linkage, and onto which is tethered (via an additional flexible linkage) an antigenic peptide which is able to bind to the peptide binding groove presented by the single chain MHC class II molecule. Such a complex provides an MHC molecule which is soluble and, because the MHC class II components and corresponding antigenic peptide are permanently linked into a single chain configuration, there is no need for complex heterodimer truncation or formation. These complexes eliminate inefficient and nonspecific peptide loading. Producing the claimed MHC:peptide complexes by recombinant methodology results in specific, high yield protein production, where the final product contains only the properly configured MHC:peptide complex of choice.

As used herein, a soluble MHC class II molecules is one that does not contain the naturally occurring membrane-associated MHC class II sequences. The soluble MHC molecules of the present invention has never been membrane-associated. Further, the soluble MHC class II molecules do not contain an amino acid sequence that acts as a transmembrane domain or as a cytoplasmic domain.

The present invention therefore provides a single chain MHC class II molecule which optionally includes an antigenic peptide covalently attached to the amino terminal portion of an  $\alpha$  or  $\beta$  chain of MHC through a peptide linkage, and the C terminal of the linked  $\alpha$  or  $\beta$  chain may be attached to the N terminal portion of another  $\alpha$  or  $\beta$  chain, thereby creating a two, three, or four domain MHC molecule. The invention further provides a multimerization domain to provide a multimeric single chain MHC

class II molecules. The invention further provides novel linkers, and multimeric MHC class II molecules that are bound to different antigenic peptides.

The amino acid sequence of each of a number of Class I and Class II proteins are known, and the genes or cDNAs have been cloned. Thus, these nucleic acids can be used to express MHC polypeptides. If a desired MHC gene or cDNA is not available, cloning methods known to those skilled in the art may be used to isolate the genes. One such method that can be used is to purify the desired MHC polypeptide, obtain a partial amino acid sequence, synthesize a nucleotide probe based on the amino acid sequence, and use the probe to identify clones that harbor the desired gene from a cDNA or genomic library.

### Linkers

Linkers of the current invention may be from about 1 to about 50 amino acids in length, depending on the molecular model of the MHC or MHC:peptide complex. In one embodiment, flexible linkers are made of repeating Gly residues separated by one or more Ser residues to permit a random, flexible motion. In the case of Class II MHC complexes this flexibility accommodates positioning of the  $\alpha$  and  $\beta$  segments to properly configure the binding groove, and also allows for maximum positioning of the peptide in the groove. In another embodiment, the linker comprises a CD4 binding site, as described below in the Example section (see also Table 1). In another embodiment, longer linkers between the chains contain flexible residues (e.g. alanine or glycine) and polar residues (e.g. serine and threonine). To inhibit the continuation of secondary structure across the linker, prolines can be added to bracket the linkers. These prolines are known to inhibit the formation of alpha helices and beta sheets. In another embodiment, flexible regions present in the human MHC and in the murine MHC could be used to make a linker by extending the region of interest and ligating the ends together. Finally, a combination of these types of linkers could also be used.

Linker position and length can be modeled based on the crystal structure of MHC Class II molecules (Brown *et al.*, *Nature* 364:33-39, 1993), where  $\alpha 1$  and  $\beta 1$  are assembled to form the peptide binding groove. Linkers joining segments of the  $\alpha$  and  $\beta$  chains together are based on the geometry of the region in the hypothetical binding site and the distance between the C terminus and the N terminus of the relevant segments.

Molecular modeling based on the X-ray crystal structure of Class II MHC (Stern *et al.*, *Nature* 368:215-221, 1994) dictates the length of linkers joining antigenic peptide,  $\alpha$  chain segments and  $\beta$  chain segments. The recombinant portions of the molecules of the invention can also be directly linked, without additional amino acids linkers.

5

### Identification of autoantigens

The invention also provides methods for preparing responder T-cell clones that proliferate when combined with a selected antigenic peptide presented by a stimulator cell. Such clones can be used to identify and map antigenic peptides associated with autoimmune disease. These peptides can then be incorporated into the single chain MHC class II molecule:peptide complexes of the invention. The method provides isolation and enrichment of non-adherent, CD56<sup>-</sup>, CD8<sup>-</sup> T cells that are reactive with a selected antigenic peptide. These cells are herein referred to as responder cells. Suitable responder cells can be isolated, for example, from peripheral blood mononuclear cells (PBMNC) obtained from patients prior to or after onset of an autoimmune disease of interest. For example, PBMNCs can be obtained from prediabetic and new onset diabetic patients. These patients can be pre-screened for specific HLA markers, such as DR3-DR4 or DQ3.2, which have the highest association with susceptibility to IDDM. From the collected PBMNCs, a portion is kept to serve as stimulator cells. From the remainder, the desired autoreactive responder cells are purified and isolated by two rounds of plating, to remove adherent cells from the population, followed by removal of monocytes and B cells with nylon wool. Enrichment for non-adherent CD4<sup>+</sup> T cells is completed by sequential plating of the cells onto plates coated with anti-CD8 and anti-CD56 antibodies.

The stimulator cells are pulsed or primed with whole GAD or an appropriate antigenic peptide. For example, stimulator cells from the PBMNCs of IDDM patients can be stimulated with antigenic GAD peptides then combined with PBMNCs or responder cells. After seven or 14 days, responder cell (T cell) clones are generated through limiting dilution and tested for antigen reactivity.

These responder cell (T cell) clones can then be used, for example, to map epitopes which bind to MHC and are recognized by a particular T cell. One such method uses overlapping peptide fragments of the autoantigen which are generated by tryptic

digestion, or more preferably, overlapping peptides are synthesized using known peptide synthesis techniques. The peptide fragments are then tested for their ability to stimulate the responder T cell clones or lines (for example, Ota *et al.*, *Nature* 346:183-187, 1990).

Once such a peptide fragment has been identified, synthetic antigenic peptides can be specifically designed, for example, to enhance the binding affinity for MHC and to out-compete any naturally processed peptides. Such synthetic peptides, when combined into a single chain MHC class II molecule:peptide complex, would allow manipulation of the immune system *in vivo*, in order to tolerize or anergize disease-associated activated T cells, thereby ameliorating the autoimmune disease.

Dissecting the functional role of individual peptides and peptide clusters in the interaction of a peptide ligand with an MHC molecule, and also in subsequent T cell recognition and reactivity, is a difficult undertaking due to the degeneracy of peptide binding to the MHC. Changes in T cell recognition or in the ability of an altered peptide to associate with MHC can be used to establish that a particular amino acid or group of amino acids comprises part of an MHC or T cell determinant. The interactions of altered peptides can be further assessed by competition with the parental peptide for presentation to a T cell, or through development of direct peptide-MHC binding assays. Changes to a peptide that do not involve MHC binding could well affect T cell recognition. For example, in a peptide, specific MHC contact points might only occur within a central core of a few consecutive or individual amino acids, whereas those amino acids involved in T cell recognition may include a completely different subset of residues.

In a preferred method, residues that alter T cell recognition are determined by substituting amino acids for each position in the peptide in question, and by assessing whether such change in residues alters the peptide's ability to associate with MHC (Allen *et al.*, *Nature* 327:713-15, 1987; Sette *et al.*, *Nature* 328:395-99, 1987; O'Sullivan *et al.*, *J. Immunol.* 147:2663-69, 1991; Evavold *et al.*, *J. Immunol.* 148:347-53, 1992; Jorgensen *et al.*, *Annu. Rev. Immunol.* 10:835-73, 1992; Hammer *et al.*, *Cell* 74:197-203, 1993; Evavold *et al.*, *Immunol. Today* 14:602-9, 1993; Hammer *et al.*, *Proc. Natl. Acad. Sci. USA* 91:4456-60, 1994; and Reich *et al.*, *J. Immunol.* 154:2279-88, 1994). One method would involve generating a panel of altered peptides wherein individual or groups of amino acid residues are substituted with conservative, semi-conservative or non-conservative residues. A preferred variant of this method is an alanine scan (Ala scan)

where a series of synthetic peptides are synthesized wherein each individual amino acid is substituted with L-alanine (L-Ala scan). Alanine is the amino acid of choice because it is found in all positions (buried and exposed), in secondary structure, it does not impose steric hindrances, or add additional hydrogen bonds or hydrophobic side chains. Alanine substitutions can be done independently or in clusters depending on the information desired. Where the information pertains to specific residues involved in binding, each residue in the peptide under investigation can be converted to alanine and the binding affinity compared to the unsubstituted peptide. Additional structural and conformational information regarding each residue and the peptide as a whole can be gained, for example, by synthesizing a series of analogs wherein each residue is substituted with a D-amino acid such as D-alanine (D-Ala scan) (Galantino *et al.*, in Smith, J. and Rivier, J. (eds.), *Peptides Chemistry and Biology (Proceedings of the Twelfth American Peptide Symposium)*, ESCOM, Leiden, 1992, pp. 404-05). Essential residues can be identified, and nonessential residues targeted for modification, deletion or replacement by other residues that may enhance a desired quality (Cunningham and Wells, *Science* 244:1081-1085, 1989; Cunningham and Wells, *Natl. Acad. Sci. USA*, 88:3407-3411, 1991; Ehrlich *et al.*, *J. Biol. Chem.* 267:11606-11, 1992; Zhang *et al.*, *Proc. Natl. Acad. Sci. USA* 90:4446-50, 1993; see also "Molecular Design and Modeling: Concepts and Applications Part A Proteins, Peptides, and Enzymes," *Methods in Enzymology*, Vol. 202, Langone (ed.), Academic Press, San Diego, CA, 1991).

Truncated peptides can be generated from the altered or unaltered peptides by synthesizing peptides wherein amino acid residues are truncated from the N- or C-terminus to determine the shortest active peptide, or between the N- and C-terminus to determine the shortest active sequence. Such peptides could be specifically developed to stimulate a response when joined to a particular MHC to form a peptide ligand to induce anergy in appropriate T cells *in vivo* or *in vitro*.

#### **Analysis of single chain MHC class II molecule:peptide complexes**

The physical and biological properties of the single chain MHC class II molecule:peptide complexes may be assessed in a number of ways. Mass spectral analysis methods such as electrospray and Matrix-Assisted Laser Desorption/Ionization Time Of Flight mass spectrometry (MALDI TOF) analysis are routinely used in the art to



provide such information as molecular weight and confirm disulfide bond formation. FACs analysis can be used to determine proper folding of the single chain complex.

An ELISA (Enzyme-linked Immunosorbent Assay) can be used to measure concentration and confirm correct folding of the single chain MHC class II molecule:peptide complexes. This assay can be used with either whole cells; solubilized MHC, removed from the cell surface; or free single chain MHC class II molecule:peptide complexes of the current invention. In an exemplary ELISA, an antibody that detects the recombinant MHC haplotype is coated onto wells of a microtiter plate. In a preferred embodiment, the antibody is L243, a monoclonal antibody that recognizes only correctly folded HLA-DR MHC dimers. One of skill in the art will recognize that other MHC Class II-specific antibodies are known and available. Alternatively, there are numerous routine techniques and methodologies in the field for producing antibodies (for example, Hurrell, (ed)., *Monoclonal Hybridoma Antibodies: Techniques and Applications*, CRC Press Inc., Boca Raton, FL, 1982), if an appropriate antibody for a particular haplotype does not exist. Anti-MHC Class II antibodies can also be used to purify Class II molecules through techniques such as affinity chromatography, or as a marker reagent to detect the presence of Class II molecules on cells or in solution. Such antibodies are also useful for Western analysis or immunoblotting, particularly of purified cell-secreted material. Polyclonal, affinity purified polyclonal, monoclonal and single chain antibodies are suitable for use in this regard. In addition, proteolytic and recombinant fragments and epitope binding domains can be used herein. Chimeric, humanized, veneered, CDR-replaced, reshaped or other recombinant whole or partial antibodies are also suitable.

In the ELISA format, bound MHC molecules can be detected using an antibody or other binding moiety capable of binding MHC molecules. This binding moiety or antibody may be tagged with a detectable label, or may be detected using a detectably labeled secondary antibody or binding reagent. Detectable labels or tags are known in the art, and include fluorescent, colorimetric and radiolabels, for instance.

Other assay strategies can incorporate specific T-cell receptors to screen for their corresponding MHC-peptide complexes, which can be done either *in vitro* or *in vivo*. For example, an *in vitro* anergy assay determines if non-responsiveness has been induced in the T cells being tested. Briefly, an MHC molecule containing antigenic peptide in the peptide binding groove can be mixed with responder cells, preferably

peripheral blood mononuclear cells (PBMN) (a heterogeneous population including B and T lymphocytes, monocytes and dendritic cells), PBMNC lymphocytes, freshly isolated T lymphocytes, *in vivo* primed splenocytes, cultured T cells, or established T cell lines or clones. Responder cells from mammals immunized with, or having a demonstrable cellular immune response to, the antigenic peptide are particularly preferred.

Subsequently, these responder cells are combined with stimulator cells (antigen presenting cells; APCs) that have been pulsed or primed with the same antigenic peptide. In a preferred embodiment, the stimulator cells are antigenic peptide-presenting cells, such as PBMNCs, PBMNCs that have been depleted of lymphocytes, appropriate antigenic peptide-presenting cell lines or clones (such as EBV-transformed B cells), EBV transformed autologous and non-autologous PMNCs, genetically engineered antigen presenting cells, such as mouse L cells or bare lymphocyte cells BLS-1, in particular, DRB1\*0401, DRB1\*0404 and DRB1\*0301 (Kovats *et al.*, *J. Exp. Med.* 179:2017-22, 1994), or *in vivo* or *in vitro* primed or pulsed splenocytes. Stimulator cells from mammals immunized with, or having a demonstrable cellular immune response to, the antigenic peptide are particularly preferred. For certain assay formats, it is preferred to inhibit the proliferation of stimulator cells prior to mixing with responder cells. This inhibition may be achieved by exposure to gamma irradiation or to an anti-mitotic agent, such as mitomycin C, for instance. Appropriate negative controls are also included (nothing; syngeneic APC; experimental peptide; APC + Peptide; MHC:peptide complex; control peptide +/- APC). Further, to assure that non-responsiveness represents anergy, the proliferation assay may be set up in duplicate, +/- recombinant IL-2 since it has been demonstrated that IL-2, can rescue anergized cells.

After an approximately 72 hour incubation, the activation of responder cells in response to the stimulator cells is measured. In a preferred embodiment, responder cell activation is determined by measuring proliferation using <sup>3</sup>H-thymidine uptake (Crowley *et al.*, *J. Immunol. Meth.* 133:55-66, 1990). Alternatively, responder cell activation can be measured by the production of cytokines, such as IL-2, or by determining the presence of responder cell-specific, and particularly T cell-specific, activation markers. Cytokine production can be assayed by testing the ability of the stimulator + responder cell culture supernatant to stimulate growth of cytokine-dependent

cells. Responder cell- or T cell-specific activation markers may be detected using antibodies specific for such markers.

Preferably, the single chain MHC class II molecule:peptide complex induces non-responsiveness (for example, anergy) in the antigenic peptide-reactive responder cells. In addition to single chain MHC class II molecule:peptide complex recognition, responder cell activation requires the involvement of co-receptors on the stimulator cell (the APC) that have been stimulated with co-stimulatory molecules. By blocking or eliminating stimulation of such co-receptors (for instance, by exposing responder cells to purified single chain MHC class II molecule:peptide complex, by blocking with anti-receptor or anti-ligand antibodies, or by "knocking out" the gene(s) encoding such receptors), responder cells can be rendered non-responsive to antigen or to single chain MHC class II molecule:peptide complex.

In a preferred embodiment, responder cells are obtained from a source manifesting an autoimmune disease or syndrome. Alternatively, autoantigen-reactive T cell clones or lines are preferred responder cells. In another preferred embodiment, stimulator cells are obtained from a source manifesting an autoimmune disease or syndrome. Alternatively, APC cell lines or clones that are able to appropriately process and/or present autoantigen to responder cells are preferred stimulator cells. In a particularly preferred embodiment, responder and stimulator cells are obtained from a source with diabetes or multiple sclerosis.

At this point, the responder T cells can be selectively amplified and/or stimulated, thereby producing a subset of T cells that are specific for the antigenic peptide. For instance, antigenic peptide-reactive responder cells may be selected by flow cytometry, and particularly by fluorescence activated cell sorting. This subset of responder cells can be maintained by repetitive stimulation with APCs presenting the same antigenic peptide. Alternatively, responder cell clones or lines can be established from this responder cell subset. Further, this subset of responder cells can be used to map epitopes of the antigenic peptide and the protein from which it is derived.

Other methods to assess the biological activity of the single chain MHC class II molecule:peptide complexes are known in the art and can be used herein, such as using a microphysiometer, to measure production of acidic metabolites in T cells following interaction with antigenic peptide. Other assay methods include competition

assays, comparing single chain MHC class II molecule:complex response with that to the normal antigen. Also measurement production of such indicators as cytokines or  $\gamma$  interferon can provide an indication of complex response.

## 5     **Animal models of autoimmune disease**

Similar assays and methods can be developed for and used in animal models. For instance, the therapeutic effect of a pharmaceutical composition of the single chain molecule or multimer or a polynucleotide encoding the single chain molecule or multimer can be tested *in vivo* in a number of animal models of HLA-DR-associated  
10     autoimmune disease. These diseases include, but are not limited to, insulin-dependent diabetes mellitus, multiple sclerosis, myasthenia gravis, pernicious anemia, rheumatoid arthritis, and systemic lupus erythematosus.

For example, NOD mice are a spontaneous model of IDDM. Treatment with the pharmaceutical compositions prior to or after onset of disease can be monitored  
15     by assay of urine glucose levels in the NOD mouse, as well as by *in vitro* T cell proliferation assays to assess reactivity to known autoantigens (*see, e.g.,* Kaufman *et al.*, *Nature* 366:69-72 (1993)) for example). Alternatively, induced models of autoimmune disease, such as EAE, can be treated with pharmaceutical composition. Treatment in a preventive or intervention mode can be followed by monitoring the clinical symptoms of  
20     EAE.

Following is a description of several other animal models of HLA-DR-associated autoimmune disease which can be used to assay *in vivo* effects of the peptide. It will be obvious to one of skill in the art that other suitable animal models for autoimmune diseases can be utilized in a similar manner.

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### Systemic Lupus Erythematosus (SLE)

F<sub>1</sub> hybrids of autoimmune New Zealand black (NZB) mice and the phenotypically normal New Zealand White (NZW) mouse strain develop severe systemic autoimmune disease, more fulminant than that found in the parental NZB strain. These  
30     mice manifest several immune abnormalities, including antibodies to nuclear antigens and subsequent development of a fatal, immune complex-mediated glomerulonephritis with

female predominance, remarkably similar to SLE in humans (Knight *et al.*, *J. Exp. Med.* 147:1653 (1978)), which is incorporated hereby by reference.

In both the human and murine forms of the disease, a strong association with MHC gene products has been reported. HLA-DR2 and HLA-DR3 individuals are at a higher risk than the general population to develop SLE (Reinertsen *et al.*, *N. Engl. J. Med.* 299:515 (1970)), while in NZB/W F<sub>1</sub> mice (H-2<sup>d/u</sup>), a gene linked to the h-2<sup>u</sup> haplotype derived from the NZW parent contributes to the development of the lupus-like nephritis.

The effect of the invention can be measured by survival rates and by the progress of development of the symptoms, such as proteinuria and appearance of anti-DNA antibodies.

Proteinuria can be measured by any method known to those of skill in the art, *e.g.* colorimetrically by the use of Uristix (Miles Laboratories, Inc., Elkhart, IN), giving an approximation of proteinuria as follows: trace, 10 mg/dl; 1+, 30 mg/dl; 100 mg/dl; 3+, 300 mg/dl; and 4+, 1000 mg/dl.

The presence of anti-DNA specific antibodies in NZB/W F<sub>1</sub> mice can be determined by using a modification of a linked immunosorbent assay (ELISA) described by Zouali *et al.*, *J. Immunol. Methods* 90:105 (1986)) which is incorporated herein by reference.

20

#### Myasthenia Gravis (MG)

Myasthenia gravis is one of several human autoimmune diseases linked to HLA-D (Safenberg, *et al.*, *Tissue Antigens* 12:136 (1978); McDevitt *et al.*, *Arth. Rheum.* 20:59 (1977)) which are incorporated herein by reference. In MG antibodies to the acetyl choline receptors (AcChoR) impair neuromuscular transmission by mediating loss of AcChoR in the postsynaptic membrane.

SJL/J female mice are a model system for human MG. In these animals, experimental autoimmune myasthenia gravis (EAMG) can be induced by immunizing the mice with soluble AcChoR protein from another species. Susceptibility to EAMG is linked in part to the MHC and has been mapped to the region within H-2 (Christadoss *et al.*, *J. Immunol.* 123:2540 (1979)).

30

AcChoR protein can purified from *Torpedo californica* and assayed according to the method of Waldor *et al.*, *Proc. Natl. Acad. Sci. USA* 80:2713 (1983), incorporated by reference. For example, emulsified AcChoR, 15 µg in complete Freund adjuvant, is injected intradermally among six sites on the back, the hind foot pads, and the base of the tail. Animals are reimmunized with this same regimen 4 weeks later.

Evaluation can be made by measurement of anti-AcChoR antibodies by any method known to those of skill in the art, *e.g.*, a microtiter ELISA assay as described in Waldor *et al.*, *supra*. In an exemplary assay, the standard reagent volume is 50 µl per well. Reagents are usually incubated in the wells for 2 hr at RT. Five µg of AcChoR diluted in bicarbonate buffer, pH 9.6, is added to each well. After incubation with AcChoR, the plates are rinsed four times with a wash solution consisting of phosphate-buffer saline containing 0.05% Tween and 0.05% NaN<sub>3</sub>. Mouse sera are diluted in 0.01M PBS (pH 7.2), 1.5 mfr MgCl<sub>2</sub>, 2.0 mM 2-mercaptoethanol, 0.05% Tween-80, 0.05% NaN<sub>3</sub> (p-Tween buffer) and incubated on the plate. After the plate is washed, beta-galactosidase-conjugated sheep anti-mouse antibody diluted in P-Tween buffer is added to each well. After a final washing, the enzyme substrate, p-nitrophenylgalactopyranoside is added to the plate, and the degree of substrate catalysis is determined from the absorbance at 405 nm after 1 hr.

Anti-AcChoR antibodies are expected to be present in the mice immunized with AcChoR as compared to nonimmunized mice. Treatment with complex is expected to significantly reduce the titer of anti-AcChoR antibodies in the immunized mice.

The effect of treatment with the invention on clinical EAMG can also be assessed by any method known to those of skill in the art. Myasthenia symptoms include a characteristic hunched posture with drooping of the head and neck, exaggerated arching of the back, splayed limbs, abnormal walking, and difficulty in righting. Mild symptoms are present after a standard stress test, and should be ameliorated by administration of complex.

#### Rheumatoid Arthritis (RA)

In humans, susceptibility to rheumatoid arthritis is associated with HLA D/DR. The immune response in mice to native type II collagen has been used to establish an experimental model for arthritis with a number of histological and pathological

features resembling human RA. Susceptibility to collagen-induced arthritis (CIA) in mice has been mapped to the H-2 I region, particularly the I-A subregion (Huse *et al.*, *Fed. Proc.* 43:1820 (1984)).

5 Mice from a susceptible strain, DEA-1 can be caused to have CIA by treatment of the mice with native type II collagen, using the technique described in Wooley *et al.*, *J. Immunol.* 134:2366 (1985), incorporated herein by reference.

In another model adjuvant arthritis in rats is an experimental model for human arthritis, and a prototype of autoimmune arthritis triggered by bacterial antigens (Holoschitz *et al.*, *Prospects of Immunology* (1986); Pearson, *Arthritis Rheum.* 7:80  
10 (1964)). The disease is the result of a cell-mediated immune response, as evidenced by its transmissibility by a clone of T cells which were reactive against the adjuvant (MT); the target self-antigen in the disease, based upon studies with the same cloned cells, appears to be part(s) of a proteoglycan molecule of cartilage.

Adjuvant disease in rats is produced as described by Pearson *supra*, *i.e.*, by  
15 a single injection of Freund's adjuvant (killed tubercle bacilli or chemical fractions of it, mineral oil, and an emulsifying agent) given into several depot sites, preferably intracutaneously or into a paw or the base of the tail. The adjuvant is given in the absence of other antigens.

The effect of the invention treatment on manifestations of the disease can  
20 be monitored by any method known to those of skill in the art. These manifestations are histopathological, and include an acute and subacute synovitis with proliferation of synovial lining cells, predominantly a mononuclear infiltration of the articular and particular tissues, the invasion of bone and articular cartilage by connective tissue pannus, and periosteal new bone formation, especially adjacent to affected joints. In severe or  
25 chronic cases, destructive changes occur, as do fibrous or bony ankylosis. These histopathological symptoms are expected to appear in control animals at about 12 days after sensitization to the Freund's adjuvant.

#### Insulin Dependent Diabetes Mellitus (IDDM)

30 IDDM is observed as a consequence of the selective destruction of insulin-secreting cells within the Islets of Langerhans of the pancreas. Involvement of the immune system in this disease is suggested by morphologic evidence of early infiltration

of the Islets by mononuclear cells, by the detection of anti-islet cell antibodies, by the high frequency of HLA-DR3 and -DR4 alleles in IDDM populations, and by clinical associations between IDDM and various autoimmune diseases. An animal model for spontaneous IDDM and thyroiditis has been developed in the BB rat. As in humans, the rat disease is controlled in part by the genes encoding the MHC antigens, is characterized by islet infiltration, and is associated with the presence of anti-islet antibodies. The I-E equivalent class II MHC antigens appear to be involved in manifestation of the autoimmune diseases in the BB rat. Biotard *et al.*, *Proc. Natl. Acad. Sci. USA* 82:6627 (1985).

In morphologic evaluation, insulinitis is characterized by the presence of mononuclear inflammatory cells within the islets. Thyroiditis is characterized by focal interstitial lymphocytic infiltrate within the thyroid gland, as a minimum criterion. Most severe cases show diffuse extensive lymphocytic infiltrates, disruption of acini, fibrosis, and focal Hurthle cell change. See Biotard *et al. supra*.

Treatment of the BB rats with the invention is expected to ameliorate or prevent the manifestation of the clinical and morphological symptoms associated with IDDM and thyroiditis.

In another model, the NOD mouse strain (H-2K<sup>d</sup> D<sup>b</sup>) is a murine model for autoimmune IDDM. The disease in these animals is characterized by anti-islet cell antibodies, severe insulinitis, and evidence for autoimmune destruction of the beta-cells (Kanazawa, *et al.*, *Diabetologia* 27:113 (1984)). The disease can be passively transferred with lymphocytes and prevented by treatment with cyclosporin-A (Ikehara *et al.*, *Proc. Natl. Acad. Sci. USA* 82:7743 (1985)); Mori *et al.*, *Diabetologia* 29:244 (1986).

Untreated animals develop profound glucose intolerance and ketosis and succumb within weeks of the onset of the disease. Seventy to ninety percent of female and 20-30% of male animals develop diabetes within the first six months of life. Breeding studies have defined at least two genetic loci responsible for disease susceptibility, one of which maps to the MHC. Characterization of NOD Class II antigens at both the serologic and molecular level suggest that the susceptibility to autoimmune disease is linked to I-A<sub>B</sub> (Acha-Orbea and McDevitt, *Proc. Natl. Acad. Sci. USA* 84:235 (1987)).

Treatment of Female NOD mice with complex is expected to lengthen the time before the onset of diabetes and/or to ameliorate or prevent the disease.



### Experimental Allergic Encephalomyelitis (EAE)

Experimental allergic encephalomyelitis (EAE) is an induced autoimmune disease of the central nervous system which is a model for multiple sclerosis (MS). The  
5 disease can be induced in many species, including mice and rats.

The disease is characterized by the acute onset of paralysis. Perivascular infiltration by mononuclear cells in the CNS is observed in both mice and rats. Methods of inducing the disease, as well as symptomology, are reviewed in Aranson, *The Autoimmune Diseases* (Rose and Mackay, eds., 1985), and in Acha-Orbea *et al.*, *Ann. Rev. Imm.* 7:377-405 (1989).  
10

One of the genes mediating susceptibility is localized in the MHC class II region (Moore *et al.*, *J. Immunol.* 124:1815-1820 (1980)). The best analyzed encephalitogenic protein is myelin basic protein (MBP), but other encephalitogenic antigens are found in the brain. The immunogenic epitopes have been mapped (*see*, Acha-  
15 Orbea *et al.*, *supra.*). In the PL mouse strains (H-2<sup>u</sup>) two encephalitogenic peptides in MBP have been characterized: MBP peptide p35-47 (MBP 35-47), and acetylated NSF p1-9 (MBP 1-9).

The effect of the invention on ameliorating disease symptoms in individuals in which EAE has been induced can be measured by survival rates, and by the  
20 progress of the development of symptoms.

### **Methods of making the complexes of the invention**

Expression systems suitable for production of appropriate recombinant single chain MHC class II molecule:peptide complexes are available and known in the  
25 art. Various prokaryotic, fungal, and eukaryotic host cells are suitable for expression of recombinant, single chain MHC class II molecule:peptide complexes, as well as for individual recombinant alpha and beta MHC class II chains.

Prokaryotes that are useful as host cells, according to the present invention, most frequently are represented by various strains of *Escherichia coli*.  
30 However, other microbial strains can also be used, such as bacilli, for example *Bacillus subtilis*, various species of *Pseudomonas*, or other bacterial strains.

According to the invention, the single chain MHC class II molecule:peptide complexes are expressed from recombinantly engineered nucleotide sequences that encode the single chain MHC class II molecule:peptide polypeptides by operably linking the engineered nucleic acid coding sequence to signals that direct gene expression in prokaryotes. A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or enhancer is operably linked to a coding sequence if it effects the transcription of the sequence. Generally, operably linked means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame.

The genes encoding the single chain MHC class II molecule:peptide complexes may be inserted into an "expression vector," "cloning vector," or "vector," terms which are used interchangeably herein and usually refer to plasmids or other nucleic acid molecules that are able to replicate in a chosen host cell. Expression vectors may replicate autonomously, or they can replicate by being inserted into the genome of the host cell, by methods well known in the art. Vectors that replicate autonomously will have an origin of replication or autonomous replicating sequence (ARS) that is functional in the chosen host cell(s).

Plasmid vectors that contain replication sites and control sequences derived from a species compatible with the chosen host are used. For example, *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived from *E. coli* species by Bolivar *et al.*, *Gene* 2:95-113, 1977. Often, it is desirable for a vector to be usable in more than one host cell, e.g., in *E. coli* for cloning and construction, and in a *Bacillus* cell for expression.

The expression vectors typically contain a transcription unit or expression cassette that contains all the elements required for the expression of the DNA encoding the MHC molecule in the host cells. A typical expression cassette contains a promoter operably linked to the DNA sequence encoding a single chain MHC class II molecule:peptide complex and a ribosome binding site. The promoter is preferably positioned about the same distance from the heterologous transcription start site as it is from the transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be accommodated without loss of promoter function.

In addition to a promoter sequence, the expression cassette can also contain a transcription termination region downstream of the structural gene to provide for efficient termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from a different gene.

5                   Commonly used prokaryotic control sequences which are defined herein to include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequences, include such commonly used promoters as the beta-lactamase (penicillinase) and lactose (lac) promoter systems (Change *et al.*, *Nature* 198:1056, 1977) and the tryptophan (trp) promoter system (Goeddel *et al.*, *Nucleic Acids* 10 *Res.* 8:4057-74, 1980) and the lambda-derived P<sub>L</sub> promoter and N-gene ribosome binding site (Shimatake *et al.*, *Nature* 292:128-32, 1981). Any available promoter system that functions in prokaryotes can be used.

                  Either constitutive or regulated promoters can be used in the present invention. Regulated promoters can be advantageous because the host cells can be grown 15 to high densities before expression of the single chain MHC class II molecule:peptide complexes is induced. High level expression of heterologous proteins slows cell growth in some situations. Regulated promoters especially suitable for use in *E. coli* include the bacteriophage lambda P<sub>L</sub> promoter, the hybrid *trp-lac* promoter (Amann *et al.*, *Gene* 25:167-78 1983; and the bacteriophage T7 promoter.

20                   For expression of single chain MHC class II molecule:peptide complexes in prokaryotic cells other than *E. coli*, a promoter that functions in the particular prokaryotic species is required. Such promoters can be obtained from genes that have been cloned from the species, or heterologous promoters can be used. For example, the hybrid *trp-lac* promoter functions in *Bacillus* in addition to *E. coli*.

25                   A ribosome binding site (RBS) is also necessary for expression of single chain MHC class II molecule:peptide complexes in prokaryotes. An RBS in *E. coli*, for example, consists of a nucleotide sequence 3-9 nucleotides in length located 3-11 nucleotides upstream of the initiation codon (Shine and Dalgarno, *Nature*, 254:34-40, 1975; Steitz, *In Biological regulation and development: Gene expression* (ed., 30 Goldberger), vol. 1, p. 349, 1979).

                  Translational coupling may be used to enhance expression. The strategy uses a short upstream open reading frame derived from a highly expressed gene native to

the translational system, which is placed downstream of the promoter, and a ribosome binding site followed after a few amino acid codons by a termination codon. Just prior to the termination codon is a second ribosome binding site, and following the termination codon is a start codon for the initiation of translation. The system dissolves secondary  
5 structure in the RNA, allowing for the efficient initiation of translation. See Squires, et. al., *J. Biol. Chem.* 263:16297-16302, 1988.

The single chain MHC class II molecule:peptide complexes can be expressed intracellularly, or can be secreted from the cell. Intracellular expression often results in high yields. However, some of the protein may be in the form of insoluble  
10 inclusion bodies. Although some of the intracellularly produced MHC polypeptides of the present invention may be active upon being harvested following cell lysis, the amount of soluble, active MHC polypeptide may be increased by performing refolding procedures using methods known to those of skill in the art (*see, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual Second Edition*, Cold Spring Harbor, NY, 1989.; Marston  
15 *et al., Bio/Technology* 2:800-804, 1985; Schoner *et al., Bio/Technology* 3:151-54, 1985). In one embodiment, for purification and refolding the cell pellet is lysed and refolded in urea-borate-DTT buffer followed by urea-borate buffer and reverse phase HPLC purification using either silica gel based Vydac (Hewlett Packard, Wilmington, DE) or  
20 polymer based Poros-R2 (PerSeptive Biosystems) resins, with bead size varying based on the scale of the culture and is described in further detail below. In one embodiment, e.g., for large scale refolding, the sample can be ultrafiltered into a urea-borate buffer to which is then added 0.2  $\mu$ M to 1 mM copper sulfate, preferably 0.2 to 20  $\mu$ M, after which folding occurs immediately.

More than one MHC:peptide complex may be expressed in a single  
25 prokaryotic cell by placing multiple transcriptional cassettes in a single expression vector, or by utilizing different selectable markers for each of the expression vectors which are employed in the cloning strategy.

A second approach for expressing the MHC:peptide complexes of the invention is to cause the polypeptides to be secreted from the cell, either into the  
30 periplasm or into the extracellular medium. The DNA sequence encoding the MHC polypeptide is linked to a cleavable signal peptide sequence. The signal sequence directs translocation of the MHC:peptide complex through the cell membrane. An example of a

suitable vector for use in *E. coli* that contains a promoter-signal sequence unit is pTA1S29, which has the *E. coli* *phoA* promoter and signal sequence *see, e.g.,* Sambrook *et al., supra*; Oka *et al., Proc. Natl. Acad. Sci. USA* 82:7212-16, 1985; Talmadge *et al., Proc. Natl. Acad. Sci. USA* 77:39892, 1980; Takahara *et al., J. Biol. Chem.* 260: 2670-74, 1985). Once again, multiple polypeptides can be expressed in a single cell for periplasmic association. Eukaryotic signal sequences are also well known to those of skill in the art, and cause the MHC:peptide complexes of the invention to be secreted into the extracellular medium.

The MHC:peptide complexes of the invention can also be produced as fusion proteins. This approach often results in high yields, because normal prokaryotic control sequences direct transcription and translation. In *E. coli*, *lacZ* fusions are often used to express heterologous proteins. Suitable vectors are readily available, such as the pUR, pEX, and pMR100 series (*see, e.g.,* Sambrook *et al., supra*). For certain applications, it may be desirable to cleave the non-MHC amino acids from the fusion protein after purification. This can be accomplished by any of several methods known in the art, including cleavage by cyanogen bromide, a protease, or by Factor X, (*see, e.g.,* Sambrook *et al., supra.*; Goeddel *et al., Proc. Natl. Acad. Sci. USA* 76:106-10, 1979; Nagai *et al., Nature* 309:810-12, 1984; Sung *et al., Proc. Natl. Acad. Sci. USA* 83:561-65, 1986). Cleavage sites can be engineered into the gene for the fusion protein at the desired point of cleavage.

Foreign genes, such as single chain MHC class II molecule:peptide complexes, can be expressed in *E. coli* as fusions with binding partners, such as glutathione-S-transferase (GST), maltose binding protein, or thioredoxin. These binding partners are highly translated and can be used to overcome inefficient initiation of translation of eukaryotic messages in *E. coli*. Fusion to such binding partner can result in high-level expression, and the binding partner is easily purified and then excised from the protein of interest. Such expression systems are available from numerous sources, such as Invitrogen Inc. (San Diego, CA) and Pharmacia LKB Biotechnology Inc. (Piscataway, NJ).

A method for obtaining recombinant proteins from *E. coli* which maintains the integrity of their N-termini has been described by Miller *et al. Biotechnology* 7:698-704 (1989). In this system, the gene of interest is produced as a C-terminal fusion to the

first 76 residues of the yeast ubiquitin gene containing a peptidase cleavage site.

Cleavage at the junction of the two moieties results in production of a protein having an intact authentic N-terminal residue.

The vectors containing the nucleic acids that code for the single chain MHC class II molecule:peptide complexes are transformed into prokaryotic host cells for expression. "Transformation" refers to the introduction of vectors containing the nucleic acids of interest directly into host cells by well known methods. The particular procedure used to introduce the genetic material into the host cell for expression of the single chain MHC class II molecule:peptide complex is not particularly critical. Any of the well known procedures for introducing foreign nucleotide sequences into host cells may be used. It is only necessary that the, particular host cell utilized be capable of expressing the gene.

Transformation methods, which vary depending on the type of the prokaryotic host cell, include electroporation; transfection employing calcium chloride, rubidium chloride calcium phosphate, or other substances; microprojectile bombardment; infection (where the vector is an infectious agent) ; and other methods. See, generally, Sambrook *et al., supra*, and Ausubel *et al., (eds.) Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., NY, 1987. Reference to cells into which the nucleic acids described above have been introduced is meant to also include the progeny of such cells. Transformed prokaryotic cells that contain expression vectors for single chain MHC class II molecule:peptide complexes are also included in the invention.

After standard transfection or transformation methods are used to produce prokaryotic cell lines that express large quantities of the single chain MHC class II molecule:peptide complex polypeptide, the polypeptide is then purified using standard techniques. See, e.g., Colley *et al., J. Chem.* 64:17619-22, 1989; and *Methods in Enzymology*, "Guide to Protein Purification", Deutscher, ed., Vol. 182 (1990). The recombinant cells are grown and the single chain MHC class II molecule:peptide complex is expressed. The purification protocol will depend upon whether single chain MHC class II molecule:peptide complex is expressed intracellularly, into the periplasm, or secreted from the cell. For intracellular expression, the cells are harvested, lysed, and the polypeptide is recovered from the cell lysate (Sambrook *et al., supra*). Periplasmic MHC polypeptide is released from the periplasm by standard techniques (Sambrook *et al.,*

*supra*). If the MHC polypeptide is secreted from the cells, the culture medium is harvested for purification of the secreted protein. The medium is typically clarified by centrifugation or filtration to remove cells and cell debris.

The MHC polypeptides can be concentrated by adsorption to any suitable resin (such as, for example, CDP-Sepharose, Asialoproteomycin-Sepharose 4B, or Q Sepharose), or by use of ammonium sulfate fractionation, polyethylene glycol precipitation, or by ultrafiltration. Other means known in the art may be equally suitable.

Further purification of the MHC polypeptides can be accomplished by standard techniques, for example, affinity chromatography, ion exchange chromatography, sizing chromatography, reverse phase HPLC, or other protein purification techniques used to obtain homogeneity. The purified proteins are then used to produce pharmaceutical compositions.

For secretion of a polypeptide or protein of interest, recombinant nucleic acid constructs of the invention may include sequences that encode signal sequences or other sequences that direct secretion. Secretory signal sequences, also called leader sequences, prepro sequences and/or pre sequences, are amino acid sequences that play a role in secretion of mature polypeptides or proteins from a cell. Such sequences are characterized by a core of hydrophobic amino acids and are typically (but not exclusively) found at the amino termini of newly synthesized proteins. The secretory signal sequence may be that of the protein of interest, or may be derived from another secreted protein (e.g., t-PA, a preferred mammalian secretory leader) or synthesized de novo. The secretory signal sequence is joined to the DNA sequence encoding a protein of the present invention in the correct reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain signal sequences may be positioned elsewhere in the DNA sequence of interest (*see, e.g.,* Welch *et al.*, U.S. Patent No. 5,037,743; Holland *et al.*, U.S. Patent No. 5,143,830). Very often the secretory peptide is cleaved from the mature protein during secretion. Such secretory peptides contain processing sites that allow cleavage of the secretory peptide from the mature protein as it passes through the secretory pathway. An example of such a processing site is a dibasic cleavage site, such as that recognized by the *Saccharomyces cerevisiae* KEX2 gene or a Lys-Arg processing site. Processing sites

may be encoded within the secretory peptide or may be added to the peptide by, for example, *in vitro* mutagenesis.

Secretory signals include the  $\alpha$  factor signal sequence (prepro sequence: Kurjan & Herskowitz, *Cell* 3:933-943, 1982; Kurjan *et al.*, U.S. Patent No. 4,546,082; 5 Brake, EP 116, 201), the *PHO5* signal sequence (Beck *et al.*, WO 86/00637), the BAR1 secretory signal sequence (MacKay *et al.*, U.S. Patent No. 4,613,572; MacKay, WO 87/002670), the *SUC2* signal sequence (Carlsen *et al.*, *Molecular and Cellular Biology* 3: 439-447, 1983), the a-1-antitrypsin signal sequence (Kurachi *et al.*, *Proc. Natl. Acad. Sci. USA* 78: 6826-6830, 1981), the a-2 plasmin inhibitor signal sequence (Tone *et al.*, *J. Biochem. (Tokyo)* 102: 1033-1042, 1987) and the tissue plasminogen activator signal sequence (Pennica *et al.*, *Nature* 301: 214-221, 1983). Alternately, a secretory signal sequence may be synthesized according to the rules established, for example, by von Heinje (*European Journal of Biochemistry* 133: 17-21, 1983; *Journal of Molecular Biology* 184: 99-105, 1985; *Nucleic Acids Research* 14: 4683-4690, 1986). Another 10 signal sequence is the synthetic signal LaC212 spx (1-47). ERLE described in WO 90/10075.

Secretory signal sequences may be used singly or may be combined. For example, a first secretory signal sequence may be used in combination with a sequence encoding the third domain of barrier (described in U.S. Patent No. 5,037,243, which is 20 incorporated by reference herein in its entirety). The third domain of barrier may be positioned in proper reading frame 3' of the DNA segment of interest or 5' to the DNA segment and in proper reading frame with both the secretory signal sequence and a DNA segment of interest.

The choice of suitable promoters, terminators and secretory signals for all 25 expression systems, is well within the level of ordinary skill in the art. Methods for expressing cloned genes in *Saccharomyces cerevisiae* are generally known in the art (see, "Gene Expression Technology," *Methods in Enzymology*, Vol. 185, Goeddel (ed.), Academic Press, San Diego, CA, 1990 and "Guide to Yeast Genetics and Molecular Biology," *Methods in Enzymology*, Guthrie and Fink (eds.), Academic Press, San Diego, 30 CA, 1991; which are incorporated herein by reference). Proteins of the present invention can also be expressed in filamentous fungi, for example, strains of the fungi *Aspergillus* (McKnight *et al.*, U.S. Patent No. 4,935,349, which is incorporated herein by reference).



Expression of cloned genes in cultured mammalian cells and in *E. coli*, for example, is discussed in detail in Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual. Second Edition*, Cold Spring Harbor, NY, 1989; which is incorporated herein by reference). As would be evident to one skilled in the art, one could express the proteins of the instant invention in other host cells such as avian, insect and plant cells using regulatory sequences, vectors and methods well established in the literature.

In yeast, suitable yeast vectors for use in the present invention include YRp7 (Struhl *et al.*, *Proc. Natl. Acad. Sci. USA* 76:1035-1039, 1978), YEpl3 (Broach *et al.*, *Gene* 8: 121-133, 1979), POT vectors (Kawasaki *et al.*, U.S. Patent No. 4,931,373, which is incorporated by reference herein), pJDB249 and pJDB219 (Beggs, *Nature* 275:104-108, 1978) and derivatives thereof. Preferred promoters for use in yeast include promoters from yeast glycolytic genes (Hitzeman *et al.*, *J. Biol. Chem.* 255: 12073-12080, 1980; Alber and Kawasaki, *J. Mol. Appl. Genet.* 1: 419-434, 1982; Kawasaki, U.S. Patent No. 4,599,311) or alcohol dehydrogenase genes (Young *et al.*, in *Genetic Engineering of Microorganisms for Chemicals*, Hollaender *et al.*, (eds.), p. 355, 1982; Ammerer, *Meth. Enzymol.* 101: 192-201, 1983). Other promoters are the TPI1 promoter (Kawasaki, U.S. Patent No. 4,599,311, 1986) and the ADH2-4<sup>c</sup> promoter (Russell *et al.*, *Nature* 304: 652-654, 1983; Irani and Kilgore, U.S. Patent Application Serial No. 07/784,653, CA 1,304,020 and EP 284 044, which are incorporated herein by reference). The expression units may also include a transcriptional terminator such as the TPI1 terminator (Alber and Kawasaki, *ibid.*).

Yeast cells, particularly cells of the genus *Pichia* or *Saccharomyces*, are a preferred host for use in producing compound of the current invention. Methods for transforming yeast cells with exogenous DNA and producing recombinant proteins therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311; Kawasaki *et al.*, U.S. Patent No. 4,931,373; Brake, U.S. Patent No. 4,870,008; Welch *et al.*, U.S. Patent No. 5,037,743; and Murray *et al.*, U.S. Patent No. 4,845,075, which are incorporated herein by reference. Transformed cells are selected by phenotype determined by a selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (e.g., leucine). A preferred vector system for use in yeast is the POT1 vector system disclosed by Kawasaki *et al.* (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. A

preferred secretory signal sequence for use in yeast is that of the *S. cerevisiae* MF $\alpha$ 1 gene (Brake, *ibid.*; Kurjan *et al.*, U.S. Patent No. 4,546,082). Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (*see, e.g.*, Kawasaki, U.S. Patent No. 4,599,311; Kingsman *et al.*, U.S. Patent No. 4,615,974; and Bitter, U.S. Patent No. 4,977,092, which are incorporated herein by reference) and alcohol dehydrogenase genes. See also U.S. Patent Nos. 4,990,446; 5,063,154; 5,139,936 and 4,661,454, which are incorporated herein by reference. Transformation systems for other yeasts, including *Hansenula polymorpha*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Ustilago maydis*, *Pichia pastoris*, *Pichia methanolica*, *Pichia guilliermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson *et al.*, *J. Gen. Microbiol.* 132:3459-65, 1986; Cregg, U.S. Patent No. 4,882,279; and Stroman *et al.*, U.S. Patent No. 4,879,231.

Other fungal cells are also suitable as host cells. For example, *Aspergillus* cells may be utilized according to the methods of McKnight *et al.*, U.S. Patent No. 4,935,349, which is incorporated herein by reference. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino *et al.*, U.S. Patent No. 5,162,228, which is incorporated herein by reference. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533, which is incorporated herein by reference.

Host cells containing DNA constructs of the present invention are then cultured to produce the heterologous proteins. The cells are cultured according to standard methods in a culture medium containing nutrients required for growth of the particular host cells. A variety of suitable media are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins, minerals and growth factors. The growth medium will generally select for cells containing the DNA construct by, for example, drug selection or deficiency in an essential nutrient which is complemented by a selectable marker on the DNA construct or co-transfected with the DNA construct.

Yeast cells, for example, are preferably cultured in a chemically defined medium, comprising a non-amino acid nitrogen source, inorganic salts, vitamins and essential amino acid supplements. The pH of the medium is preferably maintained at a pH greater than 2 and less than 8, preferably at pH 6.5. Methods for maintaining a stable

pH include buffering and constant pH control, preferably through the addition of sodium hydroxide. Preferred buffering agents include succinic acid and Bis-Tris (Sigma Chemical Co., St. Louis, MO). Yeast cells having a defect in a gene required for asparagine-linked glycosylation are preferably grown in a medium containing an osmotic stabilizer. A preferred osmotic stabilizer is sorbitol supplemented into the medium at a concentration between 0.1 M and 1.5 M, preferably at 0.5 M or 1.0 M. Cultured mammalian cells are generally cultured in commercially available serum-containing or serum-free media. Selection of a medium appropriate for the particular host cell used is within the level of ordinary skill in the art.

Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection (Wigler *et al.*, *Cell* 14:725, 1978; Corsaro and Pearson, *Somatic Cell Genetics* 7:603, 1981; Graham and Van der Eb, *Virology* 52:456, 1973), electroporation (Neumann *et al.*, *EMBO J.* 1:841-45, 1982) and DEAE-dextran mediated 'transfection (Ausubel *et al.*, (eds), *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., NY, 1987), which are incorporated herein by reference. Cationic lipid transfection using commercially available reagents, including the Boehringer Mannheim TRANSFECTION-REAGENT (N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethyl ammoniummethylsulfate; Boehringer Mannheim, Indianapolis, IN) or LIPOFECTIN reagent (N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride and dioleoyl phosphatidylethanolamine; GIBCO-BRL, Gaithersburg, MD) using the manufacturer-supplied directions, may also be used. A preferred mammalian expression plasmid is Zem229R (deposited under the terms of the Budapest Treaty with American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD on September 28, 1993 as an *E. coli* HB101 transformant and assigned Accession Number 69447). The production of recombinant proteins in cultured mammalian cells is disclosed, for example, by Levinson *et al.*, U.S. Patent No. 4,713,339; Hagen *et al.*, U.S. Patent No. 4,784,950; Palmiter *et al.*, U.S. Patent No. 4,579,821; and Ringold, U.S. Patent No. 4,656,134, which are incorporated herein by reference. Preferred cultured mammalian cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC No. CRL 1651), BHK (ATCC No. CRL 1632), BHK 570 (ATCC No. CRL 10314), DG44, and 293 (ATCC No. CRL 1573; Graham *et al.*, *J. Gen. Virol.* 36:59-72, 1977) cell lines. Additional suitable cell lines are known in the art and available from

public depositories such as the American Type Culture Collection, Rockville, Maryland. In general, strong transcription promoters are preferred, such as promoters from SV-40 or cytomegalovirus. *See, e.g.*, U.S. Patent No. 4,956,288. Other suitable promoters include those from metallothionein genes (U.S. Patents Nos. 4,579,821 and 4,601,978, which are  
5 incorporated herein by reference) and the adenovirus major late promoter.

Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been inserted. Such cells are commonly referred to as “transfectants.” Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as “stable  
10 transfectants.” A preferred selectable marker is a gene encoding resistance to the antibiotic neomycin. Selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems may also be used to increase the expression level of the gene of interest, a process referred to as “amplification.” Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent  
15 and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. A preferred amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (e.g. hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used.

20 The soluble, fused MHC:peptide complexes of the present invention can be purified by first isolating the polypeptides from the cells followed by conventional purification methods, such as by ion-exchange and partition chromatography as described by, for example, Coy *et al.* (Peptides Structure and Function, Pierce Chemical Company, Rockford, IL, pp 369-72, 1983) or by reverse-phase chromatography as described, for  
25 example, by Andreu and Merrifield (*Eur. J. Biochem.* 164: 585-90, 1987), or by HPLC as described, for example, by Kofod *et al.* (*Int. J. Peptide and Protein Res.* 32.: 436-40, 1988). Additional purification can be achieved by additional conventional purification means, such as liquid chromatography, gradient centrifugation, and gel electrophoresis, among others. Methods of protein purification are known in the art (see generally,  
30 Scopes, R., *Protein Purification*, Springer-Verlag, NY, 1982, which is incorporated by reference herein) and can be applied to the purification of the recombinant polypeptides described herein. Single chain MHC class II molecule:peptide complexes of at least

about 50% purity are preferred, at least about 70-80% purity more preferred, and about 95-99% or more purity most preferred, particularly for pharmaceutical uses. Once purified, either partially or to homogeneity, as desired, the single chain MHC class II molecule:peptide complexes may then be used diagnostically or therapeutically, as further  
5 described below.

### **Methods of using single chain MHC class II molecule:peptide complexes**

The single chain MHC class II molecule:peptide complexes of the present invention may be used within methods for down-regulating parts of the immune system  
10 that are reactive in autoimmune diseases. The single chain MHC class II molecule:peptide complexes of the present invention are contemplated to be advantageous for use as immunotherapeutics to induce immunological tolerance or nonresponsiveness (anergy) in patients predisposed to mount or already mounting an immune response those particular autoantigens. A patient having or predisposed to a  
15 particular autoimmune disease is identified and MHC type is determined by methods known in the art. The patient's T cells can be examined *in vitro* to determine autoantigenic peptide(s) recognized by the patient's autoreactive T cells using complexes and methods described herein. The patient can then be treated with complexes of the invention. Such methods will generally include administering single chain MHC class II  
20 molecule:peptide complex in an amount sufficient to lengthen the time period before onset of the autoimmune disease and/or to ameliorate or prevent that disease. Single chain MHC class II molecule:peptide complexes of the present invention are therefore contemplated to be advantageous for use in both therapeutic and diagnostic applications related to auto immune diseases.

25 Kits can also be supplied for therapeutic or diagnostic uses. Thus, the subject composition of the present invention may be provided, usually in a lyophilized form, in a container. The single chain MHC class II molecule:peptide complex is included in the kits with instructions for use, and optionally with buffers, stabilizers, biocides, and inert proteins. Generally, these optional materials will be present at less  
30 than about 5% by weight, based on the amount of single chain MHC class II molecule:peptide complex, and will usually be present in a total amount of at least about 0.001% by weight, based on the single chain MHC class II molecule:peptide complex

concentration. It may be desirable to include an inert extender or excipient to dilute the active ingredients, where the excipient may be present in from about 1 to 99% weight of the total composition.

Within one aspect of the present invention, single chain MHC class II molecule:peptide complexes are utilized to prepare antibodies for diagnostic or therapeutic uses. As used herein, the term "antibodies" includes polyclonal antibodies, monoclonal antibodies, antigen-binding fragments thereof such as  $F(ab')_2$  and Fab fragments, as well as recombinantly produced binding partners. These binding partners incorporate the variable or CDR regions from a gene which encodes a specifically binding antibody. The affinity of a monoclonal antibody or binding partner may be readily determined by one of ordinary skill in the art (see, Scatchard, *Ann. NY Acad. Sci.* 51: 660-72, 1949)

Methods for preparing polyclonal and monoclonal antibodies have been well described in the literature (see, for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor, NY, 1989; and Hurrell, J. G. R., Ed., *Monoclonal Hybridoma Antibodies: Techniques and Applications*, CRC Press, Ind., Boca Raton, FL, 1982, which is incorporated herein by reference). As would be evident to one of ordinary skill in the art, polyclonal antibodies may be generated from a variety of warm-blooded animals, such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, or rats, for example. The immunogenicity of the single chain MHC class II molecule:peptide complexes may be increased through the use of an adjuvant, such as Freund's complete or incomplete adjuvant. A variety of assays known to those skilled in the art may be utilized to detect antibodies which specifically bind to a single chain MHC class II molecule:peptide complex. Exemplary assays are described in detail in *Antibodies: A Laboratory Manual*, Harlow and Lane (Eds.), Cold Spring Harbor Laboratory Press, 1988. Representative examples of such assays include: concurrent immunoelectrophoresis, radio-immunoassays, radio-immunoprecipitations, enzyme-linked immuno-sorbent assays, dot blot assays, inhibition or competition assays, and sandwich assays.

Additional techniques for the preparation of monoclonal antibodies may be utilized to construct and express recombinant monoclonal antibodies. Briefly, mRNA is isolated from a B cell population and used to create heavy and light chain

immunoglobulin cDNA expression libraries in a suitable vector such as the  $\lambda$ IMMUNOZAP(H) and  $\lambda$ IMMUNOZAP(L) vectors, which may be obtained from Stratagene Cloning Systems (La Jolla, CA). These vectors are then screened individually or are co-expressed to form Fab fragments or antibodies (Huse *et al.*, *Science* 246 1275-81, 1989; Sastry *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 5728-32, 1989). Positive plaques are subsequently converted to a non-lytic plasmid which allows high level expression of monoclonal antibody fragments in *E. coli*.

Antibodies of the present invention may be produced by immunizing an animal selected from a wide variety of warm-blooded animals, such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, and rats, with a recombinant single chain MHC class II molecule:peptide complex. Serum from such animals are a source of polyclonal antibodies. Alternatively antibody producing cells obtained from the immunized animals are immortalized and screened. As the generation of human monoclonal antibodies to a human antigen, such as a single chain MHC class II molecule:peptide complex, may be difficult with conventional immortalization techniques, it may be desirable to first make non-human antibodies. Using recombinant DNA techniques, the antigen binding regions of the non-human antibody is transferred to the corresponding site of a human antibody coding region to produce a substantially human antibody molecules. Such methods are generally known in the art and are described in, for example, U.S. Patent No. 4,816,397, and EP publications 173,494 and 239,400, which are incorporated herein by reference.

In another aspect of the invention, the single chain MHC class II molecule:peptide complexes can be used to clone T cells which have specific receptors for the single chain MHC class II molecule:peptide complex. Once the single chain MHC class II molecule:peptide complex-specific T cells are isolated and cloned using techniques generally available to the skilled artisan, the T cells or membrane preparations thereof can be used to immunize animals to produce antibodies to the single chain MHC class II molecule:peptide complex receptors on T cells. The antibodies can be polyclonal or monoclonal. If polyclonal, the antibodies can be murine, lagomorph, equine, ovine, or from a variety of other mammals. Monoclonal antibodies will typically be murine in origin, produced according to known techniques, or human, as described above, or combinations thereof, as in chimeric or humanized antibodies. The anti- single chain

MHC class II molecule:peptide complex receptor antibodies thus obtained can then be administered to patients to reduce or eliminate T cell subpopulations that display such receptor. This T-cell population recognizes and participates in the immunological destruction of cells bearing the autoantigenic peptide in an individual predisposed to or  
5 already suffering from a disease, such as an autoimmune disease related to the autoantigenic peptide.

The coupling of antibodies to solid supports and their use in purification of proteins is well known in the literature (*see, for example, Methods in Molecular Biology. Vol. 1*, Walker (Ed.), Humana Press, New Jersey, 1984, which is incorporated by  
10 reference herein in its entirety). Antibodies of the present invention may be used as a marker reagent to detect the presence of MHC heterodimer:peptide complexes on cells or in solution. Such antibodies are also useful for Western analysis or immunoblotting, particularly of purified cell-secreted material. Polyclonal, affinity purified polyclonal, monoclonal and single chain antibodies are suitable for use in this regard. In addition,  
15 proteolytic and recombinant fragments and epitope binding domains can be used herein. Chimeric, humanized, veneered, CDR-replaced, reshaped or other recombinant whole or partial antibodies are also suitable.

### Pharmaceutical compositions

20 Pharmaceutically acceptable carriers are determined in part by the particular composition being administered (*e.g.*, nucleic acid, protein) as by the particular method used to administer the composition. Accordingly, there are a wide variety of suitable formulations of pharmaceutical compositions of the present invention (*see, e.g., Remington's Pharmaceutical Sciences* (17<sup>th</sup> ed., 1989). Administration can be in any  
25 convenient manner, *e.g.*, by injection, oral administration, inhalation, or transdermal application.

Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the packaged nucleic acid or polypeptide suspended in diluents, such as water, saline or PEG 400; (b) capsules, sachets or tablets,  
30 each containing a predetermined amount of the active ingredient, as liquids, solids, granules or gelatin; (c) suspensions in an appropriate liquid; and (d) suitable emulsions. Tablet forms can include one or more of lactose, sucrose, mannitol, sorbitol, calcium



phosphates, corn starch, potato starch, microcrystalline cellulose, gelatin, colloidal silicon dioxide, talc, magnesium stearate, stearic acid, and other excipients, colorants, fillers, binders, diluents, buffering agents, moistening agents, preservatives, flavoring agents, dyes, disintegrating agents, and pharmaceutically compatible carriers. Lozenge forms can  
5 comprise the active ingredient in a flavor, *e.g.*, sucrose, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin or sucrose and acacia emulsions, gels, and the like containing, in addition to the active ingredient, carriers known in the art.

The compound of choice, alone or in combination with other suitable  
10 components, can be made into aerosol formulations (*i.e.*, they can be “nebulized”) to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

Formulations suitable for parenteral administration, such as, for example,  
by intraarticular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal,  
15 and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In the practice of this invention, compositions can  
20 be administered, for example, by intravenous infusion, orally, topically, intraperitoneally, intravesically or intrathecally. Parenteral administration and intravenous administration are the preferred methods of administration. The formulations of compounds can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials.

Injection solutions and suspensions can be prepared from sterile powders,  
25 granules, and tablets of the kind previously described. Cells transduced by nucleic acids for *ex vivo* therapy can also be administered intravenously or parenterally as described above.

The polypeptides of the invention are administered prophylactically or to an individual already suffering from the disease. The compositions are administered to a  
30 patient in an amount sufficient to elicit an effective immune response. An amount adequate to accomplish this is defined as “therapeutically effective dose” or “immunogenically effective dose.” Amounts effective for this use will depend on, *e.g.*,

the peptide composition, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician, but generally range for the initial immunization dose (that is for therapeutic or prophylactic administration) from about 0.01 mg to about 50 mg per 70 kilogram patient, more commonly from about 0.5-1 mg to about 10-15 mg per 70 kg of body weight. Boosting dosages are typically from about 0.01 mg to about 50 mg of peptide, more commonly about 0.5-1 mg to about 10-15 mg, using a boosting regimen over weeks to months depending upon the patient's response and condition. A suitable protocol would include injection at time 0, 2, 6, 8, 10 and 14 weeks, followed by booster injections at 24 and 28 weeks. Booster injections can be from one, two, three, four, five or more. Initial and booster injection amounts and timing are determined based on the judgment of the physician and the antigen being administered. In one embodiment, the initial and booster dose is 1.3 mg, 4 mg, or 13 mg, administered via intramuscular injection, with at least one and up to 3 booster injections at 8 week intervals, or at least one and up to 4 booster injections at 6 week intervals.

The therapeutic methods of the present invention may involve oral tolerance (Weiner *et al.*, *Nature* 376: 177-80, 1995), or intravenous tolerance, for example. Tolerance can be induced in mammals, although conditions for inducing such tolerance will vary according to a variety of factors. To induce immunological tolerance in an adult susceptible to or already suffering from an autoantigen-related disease such as IDDM, the precise amounts and frequency of administration will also vary. For instance for adults about 20-80  $\mu\text{g/kg}$  can be administered by a variety of routes, such as parenterally, orally, by aerosols, intradermal injection, and the like. For neonates, tolerance can be induced by parenteral injection or more conveniently by oral administration in an appropriate formulation. The precise amount administered, and the mode and frequency of dosages, will vary.

The single chain MHC class II molecule:peptide complexes will typically be more tolerogenic when administered in a soluble form, rather than in an aggregated or particulate form. Persistence of a single chain MHC class II molecule:peptide complex of the invention is generally needed to maintain tolerance in an adult, and thus may require more frequent administration of the complex, or its administration in a form which

extends the half-life of the complex. See for example, Sun *et al.*, *Proc. Natl. Acad. Sci. USA* 91: 10795-99, 1994.

Within another aspect of the invention, a pharmaceutical composition is provided which comprises a single chain MHC class II molecule:peptide complex of the present invention contained in a pharmaceutically acceptable carrier or vehicle for parenteral, topical, oral, or local administration, such as by aerosol or transdermally, for prophylactic and/or therapeutic treatment, according to conventional methods. The composition may typically be in a form suited for systemic injection or infusion and may, as such, be formulated with sterile water or an isotonic saline or glucose solution.

Formulations may further include one or more diluents, fillers, emulsifiers, preservatives, buffers, excipients, and the like, and may be provided in such forms as liquids, powders, emulsions, suppositories, liposomes, transdermal patches and tablets, for example.

Pharmaceutical compositions of the present invention are administered at daily to weekly intervals. An "effective amount" of such a pharmaceutical composition is an amount that provides a clinically significant decrease in a deleterious T cell-mediated immune response to an autoantigen, for example, those associated with IDDM, or provides other pharmacologically beneficial effects. Such amounts will depend, in part, on the particular condition to be treated, age, weight, and general health of the patient, and other factors evident to those skilled in the art. Preferably the amount of the single chain MHC class II molecule:peptide complex administered will be within the range of 20-80 µg/kg. Compounds having significantly enhanced half-lives may be administered at lower doses or less frequently.

#### Adjuvants

An immunostimulant refers to essentially any substance that enhances or potentiates an immune response (antibody and/or cell-mediated) to an exogenous antigen. One preferred type of immunostimulant comprises an adjuvant. Many adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bordetella pertussis* or *Mycobacterium tuberculosis* derived proteins. Certain adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company,

Inc., Rahway, NJ); AS-2 (SmithKline Beecham, Philadelphia, PA); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF, interleukin-2, -7, -12, and other like growth factors, may also be used as adjuvants.

The compositions may also include a *Mycobacterium* species CWS adjuvant, as described above. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against the immunogenic peptide.

Certain adjuvants for eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A, together with an aluminum salt. MPL<sup>®</sup> adjuvants are available from Corixa Corporation (Seattle, WA; *see*, for example, US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555, WO 99/33488 and U.S. Patent Nos. 6,008,200 and 5,856,462. Immunostimulatory DNA sequences are also described, for example, by Sato *et al.*, *Science* 273:352, 1996. Another preferred adjuvant comprises a saponin, such as Quil A, or derivatives thereof, including QS21 and QS7 (Aquila Biopharmaceuticals Inc., Framingham, MA); Escin; Digitonin; or *Gypsophila* or *Chenopodium quinoa* saponins. Other preferred formulations include more than one saponin in the adjuvant combinations of the present invention, for example combinations of at least two of the following group comprising QS21, QS7, Quil A,  $\beta$ -escin, or digitonin.

Alternatively the saponin formulations may be combined with vaccine vehicles composed of chitosan or other polycationic polymers, polylactide and polylactide-co-glycolide particles, poly-N-acetyl glucosamine-based polymer matrix, particles composed of polysaccharides or chemically modified polysaccharides, liposomes and lipid-based particles, particles composed of glycerol monoesters, etc. The saponins may also be formulated in the presence of cholesterol to form particulate structures such as liposomes or ISCOMs. Furthermore, the saponins may be formulated together with a polyoxyethylene ether or ester, in either a non-particulate solution or

suspension, or in a particulate structure such as a paucilamellar liposome or ISCOM. The saponins may also be formulated with excipients such as Carbopol<sup>®</sup> to increase viscosity, or may be formulated in a dry powder form with a powder excipient such as lactose.

In one embodiment, the adjuvant system includes the combination of a  
5 monophosphoryl lipid A and a saponin derivative, such as the combination of QS21 and 3D-MPL<sup>®</sup> adjuvant, as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other formulations comprise an oil-in-water emulsion and tocopherol. Another adjuvant formulation employs QS21, 3D-MPL<sup>®</sup> adjuvant and tocopherol in an oil-in-water  
10 emulsion is described in WO 95/17210.

Another enhanced adjuvant system involves the combination of a CpG-containing oligonucleotide and a saponin derivative particularly the combination of CpG and QS21 as disclosed in WO 00/09159. Preferably the formulation additionally comprises an oil in water emulsion and tocopherol.

15 Additional illustrative adjuvants for use in the pharmaceutical compositions of the invention include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (*e.g.*, SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Enhanzyn<sup>®</sup>) (Corixa, Hamilton, MT), RC-529 (Corixa, Hamilton, MT)  
20 and other aminoalkyl glucosaminide 4-phosphates (AGPs), such as those described in pending U.S. Patent Application Serial Nos. 08/853,826 and 09/074,720, the disclosures of which are incorporated herein by reference in their entireties, and polyoxyethylene ether adjuvants such as those described in WO 99/52549A1.

Other preferred adjuvants include adjuvant molecules of the general  
25 formula (I):  $\text{HO}(\text{CH}_2\text{CH}_2\text{O})_n\text{-A-R}$ ,  
wherein,  $n$  is 1-50,  $A$  is a bond or  $-\text{C}(\text{O})-$ ,  $R$  is  $\text{C}_{1-50}$  alkyl or Phenyl  $\text{C}_{1-50}$  alkyl.

One embodiment of the present invention consists of a vaccine formulation comprising a polyoxyethylene ether of general formula (I), wherein  $n$  is between 1 and 50, preferably 4-24, most preferably 9; the  $R$  component is  $\text{C}_{1-50}$ , preferably  $\text{C}_4\text{-C}_{20}$  alkyl  
30 and most preferably  $\text{C}_{12}$  alkyl, and  $A$  is a bond. The concentration of the polyoxyethylene ethers should be in the range 0.1-20%, preferably from 0.1-10%, and most preferably in the range 0.1-1%. Preferred polyoxyethylene ethers are selected from the following

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group: polyoxyethylene-9-lauryl ether, polyoxyethylene-9-stearyl ether, polyoxyethylene-8-stearyl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether. Polyoxyethylene ethers such as polyoxyethylene lauryl ether are described in the Merck index (12<sup>th</sup> edition: entry 7717). These adjuvant molecules are described in WO 99/52549.

The polyoxyethylene ether according to the general formula (I) above may, if desired, be combined with another adjuvant. For example, a preferred adjuvant combination is preferably with CpG as described in the pending UK patent application GB 9820956.2.

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#### Liposome-, Nanocapsule-, and Microparticle-Mediated Delivery

In certain embodiments, the inventors contemplate the use of liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, and the like, for the introduction of the compositions of the present invention into the subjects. In particular, the compositions of the present invention may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like.

Such formulations may be preferred for the introduction of pharmaceutically-acceptable formulations of the compositions disclosed herein. The formation and use of liposomes is generally known to those of skill in the art (*see*, for example, Couvreur *et al.*, 1977; Couvreur, 1988; Lasic, 1998; which describes the use of liposomes and nanocapsules in the targeted antibiotic therapy for intracellular bacterial infections and diseases). Recently, liposomes were developed with improved serum stability and circulation half-times (Gabizon & Papahadjopoulos, 1988; Allen and Choun, 1987; U. S. Patent 5,741,516, specifically incorporated herein by reference in its entirety). Further, various methods of liposome and liposome-like preparations as potential drug carriers have been reviewed (Takakura, 1998; Chandran *et al.*, 1997; Margalit, 1995; U. S. Patent 5,567,434; U. S. Patent 5,552,157; U. S. Patent 5,565,213; U. S. Patent 5,738,868 and U. S. Patent 5,795,587, each specifically incorporated herein by reference in its entirety).

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Liposomes have been used effectively to introduce genes, drugs (Heath & Martin, 1986; Heath *et al.*, 1986; Balazsovits *et al.*, 1989; Fresta & Puglisi, 1996),

radiotherapeutic agents (Pikul *et al.*, 1987), enzymes (Imaizumi *et al.*, 1990a; Imaizumi *et al.*, 1990b), viruses (Faller & Baltimore, 1984), transcription factors and allosteric effectors (Nicolau & Gersonde, 1979) into a variety of cultured cell lines and animals. In addition, several successful clinical trials examining the effectiveness of liposome-mediated drug delivery have been completed (Lopez-Berestein *et al.*, 1985a; 1985b; Coune, 1988; Sculier *et al.*, 1988). Furthermore, several studies suggest that the use of liposomes is not associated with autoimmune responses, toxicity or gonadal localization after systemic delivery (Mori & Fukatsu, 1992).

Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs). MLVs generally have diameters of from 25 nm to 4  $\mu$ m. Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 Å, containing an aqueous solution in the core.

Liposomes bear resemblance to cellular membranes and are contemplated for use in connection with the present invention as carriers for the compositions. They are widely suitable as both water- and lipid-soluble substances can be entrapped, *i.e.*, in the aqueous spaces and within the bilayer itself, respectively. It is possible that the drug-bearing liposomes may even be employed for site-specific delivery of active agents by selectively modifying the liposomal formulation.

In addition to the teachings of Couvreur *et al.* (1977, 1988), the following information may be utilized in generating liposomal formulations. Phospholipids can form a variety of structures other than liposomes when dispersed in water, depending on the molar ratio of lipid to water. At low ratios the liposome is the preferred structure. The physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations. Liposomes can show low permeability to ionic and polar substances, but at elevated temperatures undergo a phase transition which markedly alters their permeability. The phase transition involves a change from a closely packed, ordered structure, known as the gel state, to a loosely packed, less-ordered structure, known as the fluid state. This occurs at a characteristic phase-transition temperature and results in an increase in permeability to ions, sugars and drugs.

In addition to temperature, exposure to proteins can alter the permeability of liposomes. Certain soluble proteins, such as cytochrome c, bind, deform and penetrate

the bilayer, thereby causing changes in permeability. Cholesterol inhibits this penetration of proteins, apparently by packing the phospholipids more tightly. It is contemplated that the most useful liposome formations for antibiotic and inhibitor delivery will contain cholesterol.

5                   The ability to trap solutes varies between different types of liposomes. For example, MLVs are moderately efficient at trapping solutes, but SUVs are extremely inefficient. SUVs offer the advantage of homogeneity and reproducibility in size distribution. However, a compromise between size and trapping efficiency is offered by large unilamellar vesicles (LUVs). These are prepared by ether evaporation and are three  
10 to four times more efficient at solute entrapment than MLVs.

                  In addition to liposome characteristics, an important determinant in entrapping compounds is the physicochemical properties of the compound itself. Polar compounds are trapped in the aqueous spaces and nonpolar compounds bind to the lipid bilayer of the vesicle. Polar compounds are released through permeation or when the  
15 bilayer is broken, but nonpolar compounds remain affiliated with the bilayer unless it is disrupted by temperature or exposure to lipoproteins. Both types show maximum efflux rates at the phase transition temperature.

                  Liposomes interact with cells *via* four different mechanisms: endocytosis by phagocytic cells of the reticuloendothelial system such as macrophages and  
20 neutrophils; adsorption to the cell surface, either by nonspecific weak hydrophobic or electrostatic forces, or by specific interactions with cell-surface components; fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal contents into the cytoplasm; and by transfer of liposomal lipids to cellular or subcellular membranes, or vice versa, without  
25 any association of the liposome contents. It often is difficult to determine which mechanism is operative and more than one may operate at the same time.

                  The fate and disposition of intravenously injected liposomes depend on their physical properties, such as size, fluidity, and surface charge. They may persist in tissues for hours or days, depending on their composition, and half lives in the blood  
30 range from minutes to several hours. Larger liposomes, such as MLVs and LUVs, are taken up rapidly by phagocytic cells of the reticuloendothelial system, but physiology of the circulatory system restrains the exit of such large species at most sites. They can exit



only in places where large openings or pores exist in the capillary endothelium, such as the sinusoids of the liver or spleen. Thus, these organs are the predominant site of uptake. On the other hand, SUVs show a broader tissue distribution but still are sequestered highly in the liver and spleen. In general, this *in vivo* behavior limits the potential targeting of liposomes to only those organs and tissues accessible to their large size. These include the blood, liver, spleen, bone marrow, and lymphoid organs.

Targeting is generally not a limitation in terms of the present invention. However, should specific targeting be desired, methods are available for this to be accomplished. Antibodies may be used to bind to the liposome surface and to direct the antibody and its drug contents to specific receptors located on a particular cell-type surface. Carbohydrate determinants (glycoprotein or glycolipid cell-surface components that play a role in cell-cell recognition, interaction and adhesion) may also be used as recognition sites as they have potential in directing liposomes to particular cell types. Mostly, it is contemplated that intravenous injection of liposomal preparations would be used, but other routes of administration are also conceivable.

Alternatively, the invention provides for pharmaceutically-acceptable nanocapsule formulations of the compositions of the present invention. Nanocapsules can generally entrap compounds in a stable and reproducible way (Henry-Michelland *et al.*, 1987; Quintanar-Guerrero *et al.*, 1998; Douglas *et al.*, 1987). To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1  $\mu\text{m}$ ) should be designed using polymers able to be degraded *in vivo*. Biodegradable polyalkylcyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention. Such particles may be easily made, as described (Couvreux *et al.*, 1980; 1988; zur Muhlen *et al.*, 1998; Zambaux *et al.* 1998; Pinto-Alphandry *et al.*, 1995 and U. S. Patent 5,145,684, specifically incorporated herein by reference in its entirety).

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that

certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

The following example is provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of noncritical parameters that could be changed or modified to yield essentially similar results.

### EXAMPLES

Example 1: Construction of DNA sequences encoding human single chain MHC class II peptide complexes, HLA-DR4 molecules(CO563 and CO564).

DNA constructs encoding gp39  $\beta 1/\alpha 1$  human molecules were prepared from a cDNA encoding the gp39 peptide fused to the  $\beta 1/\alpha 1$  domains of HLA-DR4 according to standard techniques. For the production of “empty”  $\beta 1/\alpha 1$  DR4 molecules, a cDNA encoding the fused  $\beta 1/\alpha 1$  domains of HLA-DR4 was prepared using cloned  $\alpha$  and  $\beta$  chains from DR4.

Amino acid sequence of the gp39- $\beta 1/\alpha 1$  HLA-DR4 human single chain molecule (linkers are shown in bold)

MGDTGRSFTLASSETGVGASGGGGSGGGGDTRPRFLEQVKHECH  
FFNGTERVRFLDRYFYHQEEYVRFDSDVGEYRAVTELGRPDAEYWNSQKDLLEQ  
KRAAVDTYCRHNYGVGESFTVQRRGGIKEEHVIIQAEFYLNPDQSGEFMFDFDG  
DEIFHVDMAKKETVWRLEEFGRFASFEAQGALANLAVDKANLEIMTKRSNYTPIT  
N\*

aa1 – aa4: leader sequence

aa5 - aa18: gp39 peptide

aa19 - aa28: linker

aa29 - aa122: HLA-DR4  $\beta 1$  domain

aa123 – aa124: linker

aa125 – aa 208: HLA-DR4  $\alpha 1$  domain

Amino acid sequence of the “empty”  $\beta 1/\alpha 1$  HLA-DR4 human single chain molecule (linkers in bold):

MGDTRPRFLEQVKHECHFFNGTERVRFLDRYFYHQEEYVRFDSDVGEYRAVTEL  
GRPDAEYWNSQRDLLEQKRAAVDTYCRHNYGVGESFTVQRRGGIKEEHVIIQAE

50

FYLNPDQSGEFMFDFDGDEIFHVDMAKKETVWRLEEFGRFASF EAQGALANIAV  
 DKANLEIMTKRSNYTPITN\*

aa 1 – aa95: HLA-DR4  $\beta$ 1 domain

aa96- aa97: linker

5 aa98- aa181: HLA-DR4  $\alpha$ 1 domain

For the production of recombinant proteins, the bacteria (pLysS) were grown in LB (containing ampicillin (50  $\mu$ g / ml) and chloramphenicol (5  $\mu$ g / ml)) at 37°C until OD 600 = 0.5. IPTG was added at the final concentration of 0.5 mM final and the bacteria were further incubated for 3 hours at 37°C with shaking. The bacteria were  
 10 centrifuged at 4°C, 4000xg for 20 min and the pellet was frozen at –80°C. The following day, the pellet was resuspended in 40 ml of lysis buffer (50 mM Tris-HCl pH 8, 50 mM NaCl, 2 mM EDTA, 1 protease inhibitor cocktail tablet, 1% Triton X100 and 1% deoxycholate), and incubated for 1 hour at 4°C under continuous agitation with a magnetic stirrer. The sample was then homogenized using a French Press with a 16,000  
 15 psi setting, and centrifuged at 4°C, 9000g for 20 min. The pellet was then resuspended in 30 ml of lysis buffer without Triton and deoxycholate and centrifuged at 4°C, 9000 g for 20 min. The new pellet was resuspended in 10 ml of 20 mM ethanolamine/6 M urea pH 10, and eventually frozen at –80°C. The recombinant protein was then purified by FPLC ion-exchange chromatography using Source 30Q anion-exchange media in an XK26/20  
 20 column using a step gradient going from 1 mM to 1 M NaCl in 20 mM ethanolamine/6M urea pH 10. Fractions were analyzed by SDS/PAGE and those corresponding to the proteins of interest are pooled and dialyzed against PBS 1X.

#### Example 2: Production of additional single chain constructs

25 Additional constructs with different composition and length of the 2<sup>nd</sup> linker (between  $\beta$ 1 and  $\alpha$ 1) were engineered by using standard techniques using CO567 as the template. Specifically, PCR primers were designed to replace the old sequence in CO567 with the new sequence. For example, to make CO581, the primers were designed with the following sequences (note these primers were phosphorylated at 5').

30 Primer 1:

5' pCACCAGGAGGAGAGCCGCCACGCCGGTCTCGCTGG

Primer 2:

51

5' pGACCACCTGGATCTGGGGACACCCGACCACGTTTC

PCR reaction (100 µl) was made of the following components: 2 µl CO567 (80 ng) as template, 2 µl each of primer 1 and primer 2 (10 µM), 2 µl of dNTP mix (20 mM each), 10 µl of 10X pfu buffer, and 80 µl of sterile water. After all the components were mixed, 2 µl of Turbo pfu (5U total ) was added, mixed and put on PCR machine. The PCR cycles has a pre-denaturation at 95°C for 30 sec, then 10 cycles of 95°C for 30°C, 60°C for 1 min, and 72°C for 7 min. Then another 22 cycles of 95°C for 30°C, 65°C for 1 min and 72°C for 7 min, followed by a final 10 min at 72°C.

The PCR mixture was digested with 2 µl of DpnI (10U) for 2h at 37°C. Then the PCR product at ~6 kb was purified from agarose gel after electrophoresis. The purified PCR product was ligated by T4 DNA ligase for 1 h at room temperature then used to transform into NovaBlue (Novagen)competent cells by standard protocol. Cells were plated on LB (+Carb) and grow overnight at 37°C.

Next day, about a dozen single colony from the transformation were randomly picked for overnight culture in 5 ml LB (+Carb) at 37°C. Plasmids from these culture were purified with Wizard Miniprep kit, and analyzed by Xho I digestion. A few plasmids that passed the Xho I digestion were further confirmed by DNA sequencing.

To express the recombinant proteins, a clone with confirmed DNA sequence was used to transform BL21(DE3)CodonPlus-RIL (Stratagene) by standard transformation protocol and plated on LB (Carb+Cam) plates overnight at 37°C. Next morning, a single colony was picked to inoculate 100 ml LB (+Carb +Cam) and the culture was grown till OD reach between 0.8-1.0, and stored overnight at 4°C. Next day, the culture was pellet down and used to inoculate into 2xYT (+Carb+Cam) at ratio of 25 ml culture per liter new media. These large cultures were grown at 37°C till OD=0.5-0.6, and IPTG was added to induce recombinant protein at 37°C for 3 h. The induced cultures were pellet and stored at -80°C till purification.

Table 1 provides a listing of various constructs made according to the invention.

52  
TABLE I

Construct	peptide	upstream linker	downstream linker
C0523	yes	GGGG	GG
C0543	none	none	GG
C0563	yes	ASGGGSGGG	GG
C0567	yes	ASGGGSGGG	TSGGGGSGGGGSSS
C0580	yes	ASGGGSGGG	GSPGGGGSGGGPGS
C0581	yes	ASGGGSGGG	GSPPGGPPGS
C0582	yes	ASGGGSGGG	GSPGGGGPGS
C0583	yes	ASGGGSGGG	TSGGGGS
C0584	yes	ASGGGSGGG	SGGSGGS
C0585	yes	ASGGGSGGG	FDAPSPLP
C0586	none	none	TSGGGGSGGGGSSS
C0587	none	none	GSPGGGGSGGGPGS
C0588	none	none	GSPPGGPPGS
C0589	none	none	GSPGGGGPGS
C0590	none	none	TSGGGGS
C0591	none	none	SGGSGGS
C0592	none	none	FDAPSPLP
C0593	yes	ASGGGSGGG	VYPEVTV
C0594	none	none	VYPEVTV
C0595	yes	ASGGGSGGG	GGGG
C0596	yes	ASGGGSGGG	GGGS
C0597	yes	ASGGGSGGG	GGSGG

Example 3: Four classes of novel linkers for MHC class II single chain molecules

Inspection of circular dichroism spectra of purified, refolded single chain constructs indicated that novel linkers could be used for the constructs. The atomic structures of the various murine and human MHC class II molecules, as determined by X-ray crystallography, indicated that these molecules have a high degree of structural similarity. The circular dichroism results were consistent with two folded molecules of clearly different secondary structure. Careful inspection of the structures reveals that the human MHC has a longer distance between chains than the equivalent murine molecule. This results led to proposing longer linkers between the chains which would contain flexible residues (e.g. alanine or glycine) and polar residues (e.g. serine and threonine). These constructs make up the first class of linkers. To inhibit the continuation of secondary structure across the linker, prolines were added to bracket the linkers. These prolines are known to inhibit the formation of alpha helices and beta sheets. These linkers make up the second class of linkers disclosed here.

Next, flexible regions present in the human MHC and in the murine MHC could be used to make a linker by extending the region of interest and ligating the ends together. These are the third class of linkers. Finally, a combination of these types of linkers could also be used. These are the fourth class of linkers.

Example 4: Human MHC class II single chain molecule with murine linkers

Another linker has been suggested based on a combination of murine and human MHC class II single chain molecules. This fusion would incorporate linker residues from the functional murine single chain MHC class II molecule and the alpha and beta chains of the human molecule. The protein sequence of the single chain molecule is provided below (the linker residues from the mouse construct are in bold):

MGDTGRSFTLASSETGVGASGGGGSGGGGDTRPRFLEQVKHECHFFNGTERVRF  
 LDRYFYHQEEYVRFDSDVGEYRAVTELGRPDAEYWNSQKDLLEQKRAAVDTYC  
 RHNYGVGESFTV**LRRLGGEDDEADHHV**IIQAEFYLNPDQSGEFMDFDGDGDEIFH  
 VDMAKKETVWRLEEFGRFASFEAQGALANIAVDKANLEIMTKRSNYTPITN\*

Further MHC class II hybrid single chain molecules may be designed by fusing other portions of the alpha chain and the beta chain together using linkers as described elsewhere. A properly folded molecule may be obtained by putting appropriate

linkers between portions of the human MHC class II which are proximal to each other as determined by visual inspection of the atomic coordinates of residues of the native MHC available in the publicly accessible protein structure database. These structures would predict possible fusion proteins which covalently attach any part of the beta chain  
5 between residues 82 to 123 or between residues 148 to 164 to portions of the alpha chain such as the N-terminal residues, residues 79 to 84, or 92 to 106. The numbering system of residues in this example corresponds to those found in the coordinates of the structure described in: DESSEN, *et al. Immunity* 7:473 (1997). Other, homologous residues could be used to create equivalent constructs for genotypic and allelic variants of these  
10 molecules e.g. equivalent residues in DR2 or such. DNAs for such hybrids would be prepared and expressed in a recombinant expression system by someone skilled in the art and could be assayed for structure and function in appropriate assays.

Example 5: Use of CD4 binding site(s) of MHC class II molecules as linkers for the  
15 production of bioactive recombinant MHC class II:peptide complexes

HLA class II molecules present antigenic peptides to the T cell receptor of the CD4+ T lymphocytes and interact with CD4 during the antigen recognition process. Native MHC class II-peptide complexes have been shown to bind to MHC class II restricted and antigen specific TCRs on a particular T cell and induce T cell  
20 nonresponsiveness. It is proposed that the CD4 binding site is important in the docking of MHC class II-peptide complex with the TCR and induce nonresponsiveness. Since the binding of CD4 to MHC class II-peptide is important in antigen presentation and/or induction of T cell nonresponsiveness, it is proposed that recombinant MHC class II-peptide molecules (truncated or whole) containing CD4 binding site will be biologically  
25 active. Furthermore, a polypeptide fragment from MHC class II which binds the CD4, when used as a linker in preparation of MHC class II-peptide truncated molecules, provides resulting recombinant molecules that will be biologically active.

The following describes the concept of different linkers. Peptide is attached to a linker 1 (L1) which is attached to N-terminus of  $\beta$ 1 domain that is linked to  
30 L2. L2 is linked to L3, which in turn is linked to N-terminus of  $\alpha$ 1 chain of MHC class II. Here L2 represents the human CD4 binding sequences. It should be noted that L2 could also be directly linked to N-terminus of  $\alpha$ 1 domain by completely deleting L3.

Specific examples of L1 and L3 are given in a examples. The sequences of L2 are given below. These sequences are applicable to most of the DR-Peptide molecules.

RNGQEEKAGVVSTGLI, RNGQETKAGVVSTGLI, YNQQEEKAGGVSTGLI,  
FRNGQEEKAGVVSTGLI, FRNGQETKAGVVSTGLI, FYNQQEEKAGGVSTGLI,  
and LNGQEEKAGMVSTGLI.

Example 6: I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.Ck construct and activity

The “full anergix” single chain molecule, mouse I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.Ck, was generated by overlap PCR using standard methodology. The molecules was expressed in 293T cells and baculoviral cells according to standard methodology, and purified according to standard methodology using affinity chromatography using goat-anti mouse antibodies. The structure of I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.Ck is shown in Figure 1.

The amino acid sequence of the I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.Ck shown in Figure 1 is as follows:

METDTLLLWVLLLWVPGSTGDFKNIVTPRTPPPASGGGGSGGGGDSEHFVVFQF  
KGE CYFTNGTQRI RSVDRYIYNREEYLRFDS DVGEYRAVTELGRPDPEYYNKQY  
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SERQNGVLNSWTDQDSK DSTYSMSSTLT LTKDEYERHNSYTCEATHKTSTSPIVK  
SFNRNEC

The *in vitro* activity of recombinant I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.Ck was tested using MBP90-101 specific IAs restricted mouse T cell clone HS1. This clone was prepared by the immunization of SJL mice with the MBP90-101 peptide, followed by cloning out CD4<sup>+</sup> T cells by limited dilution techniques. These cells were maintained by stimulation every 10 days with irradiated SJF splenocytes and PBP90-101 peptide. The T cells are activated by a combination of soluble recombinant I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.Ck and plate bound anti-CD28 antibody. T cell activation was assayed by <sup>3</sup>H-thymidine



incorporation according to standard methodology. Figure 2 shows the results of this assay.

MS is a T cell dependent autoimmune disease caused by localized demyelination in the central nervous system. Experimental autoimmune encephalomyelitis is a accepted animal model of MS. The following results demonstrate that administration of I-As MBP. $\beta 1\beta 2\alpha 1\alpha 2$ .C $\kappa$  reduces the incidence and severity of EAE. EAE was induced according to standard methodology according to the myelin model. Ten  $\mu$ g of recombinant I-As MBP. $\beta 1\beta 2\alpha 1\alpha 2$ .C $\kappa$  was given intravenously in 100  $\mu$ l of PBS at days 1, 4, 7, and 11 after disease induction. 12 days after immunization, animals are observed daily for the onset of neurological dysfunction. Disease is graded by trained technicians according to standard methods (see Figure 3). Mice are followed for up to 60-70 days. The data shown in Figure 4 demonstrate that administration of the recombinant I-As MBP. $\beta 1\beta 2\alpha 1\alpha 2$ .C $\kappa$  significantly reduced the incidence of myelin-induced EAE in SJL mice. In one experiment, 11 out of 20 mice developed EAE in the untreated group (55%), whereas only 2 out of 21 animal showed sign of the disease in the treated group (16.6%). Similarly, in another experiment, 12 out of 16 mice developed EAE in the untreated group (75%), while only 2 out of 16 developed EAE in the treated group (12.5%).

Histological examination from SJL mice in the EAE model studies: spinal cords were removed, fixed in formalin solution and embedded in paraffin. Sections were cut, stained with hematoxylin, eosin and graded for inflammatory lesions. A. Section of spinal cord from untreated mouse, score = 2.5; B. Section of spinal cord from mouse treated with the recombinant I-AS.MBP.C $\kappa$ , score = 0; C. Section of spinal cord from mouse treated with the recombinant I-AS. $\beta 1\alpha 1$  without antigenic peptide fusion, score = 2.0; D. Section of spinal cord from mouse treated with the recombinant I-AS.MBP. $\beta 1\alpha 1$ , score = 0.5. The histology score for each section is marked.

Example 7: Functional human anergic molecules optimized for *E. coli* expression with *E. coli* codons

Two human single chain MHC class II molecules (CO528-AC and CO608-AC) have been optimized for *E. coli* expression using "artificial codons," e.g.,

preferred *E. coli* codons encoding the mammalian protein. CO528-AC and CO608-AC were made according to standard PCR overlap technology.

Example 7: Recombinant MHC class II IAs.MBP.Ig multimeric complexes

5                   Recombinant MHC class II (IAs)-peptide-Ig fusion complexes were constructed by fusing the mIgG leader, MBP 90-101, or MBP1-14 (as a control) to IAs single chain (b1b2a1a2), and mIgG.Ck, mIgG.CH1.H, mIgG.CH1.H.CH2, or mIgG.CH1.H.CH2.CH3 with flexible linkers, according to standard methodology. The recombinant IAs fusion proteins were expressed in both mammalian and insect cells and  
10                   detected by western analysis and ELISA. The overexpressed and secreted recombinant IAs fusion proteins from both human 293 cell cultures or from insect culture medium were purified by affinity chromatography. The purified dimeric and tetrameric recombinant IAs proteins have *in vitro* biological activity as assayed using an antigen-specific mouse T cell clone. The *in vivo* activity of the recombinant IAs fusion proteins  
15                   were studied with the experimental autoimmune encephalomyelitis (EAE) model using susceptible SJL mice. In these EAE studies, recombinant IAs fusion protein was delivered on days 1, 4, 7, and 11 by I.V. injections after induction of the disease with myelin. The animals were then examined for neurological dysfunction. The results indicate that treatment with the recombinant IAs fusion proteins prevents mortality and  
20                   significantly reduces paralysis induced by myelin homogenate in CFA. In conclusion, these studies suggest that the recombinant MHC class II fusion protein has therapeutic benefit as antigen-specific drugs for the treatment of autoimmune diseases.

Example 8: Synthesis of mouse model equivalent of CO608

25                   Four forms of the murine MHC Class II IAs  $\beta 1\alpha 1$  with MBP peptide linkers analogous to CO608 human were made. These constructs can be used, e.g., as murine clinical control for human 608. mCO608 (mouse CO608, lacking the first five amino acids, GDSEK, as compared to native beta 1 domain), MCO608-A (same as m608 except lacking four amino acids, GSEK, after the methionine as compared to m608), *B. megaterium*-mCO608-A (expressed in *Bacillus megaterium*), and mCO608-B (same as  
30                   m608 except lacking first four amino acids, GSEK, after the methionine as compared to m608; also lacking second amino acid, D, as compared to native beta 1 domain).

58

To make mCO608, the upstream linker of mouse CO521 (GGGS) was replaced with the human CO608 linker (ASGGGGSGGG) and the downstream linker of mouse CO521 (GG) was replaced with the downstream linker of CO608 (TSGGGGS), using PCR according to standard methodology. mCO608-A and m6O8-B were made  
5 from mCO608 using PCR according to standard methodology.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

10 Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

59

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50     Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys
           5          10          15

      His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe
           20          25          30

55     Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr
           35          40          45

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	Arg	Ala	Val	Thr	Glu	Leu	Gly	Arg	Pro	Asp	Ala	Glu	Tyr	Trp	Asn	Ser
	50						55					60				
5	Gln	Lys	Asp	Leu	Leu	Glu	Gln	Lys	Arg	Ala	Ala	Val	Asp	Thr	Tyr	Cys
	65					70					75					80
	Arg	His	Asn	Tyr	Gly	Val	Gly	Glu	Ser	Phe	Thr	Val	Gln	Arg	Arg	Gly
					85					90					95	
10	Gly	Gly	Gly	Ser	Ile	Lys	Glu	Glu	His	Val	Ile	Ile	Gln	Ala	Glu	Phe
				100					105					110		
	Tyr	Leu	Asn	Pro	Asp	Gln	Ser	Gly	Glu	Phe	Met	Phe	Asp	Phe	Asp	Gly
15			115					120					125			
	Asp	Glu	Ile	Phe	His	Val	Asp	Met	Ala	Lys	Lys	Glu	Thr	Val	Trp	Arg
	130						135					140				
20	Leu	Glu	Glu	Phe	Gly	Arg	Phe	Ala	Ser	Phe	Glu	Ala	Gln	Gly	Ala	Leu
	145					150					155					160
	Ala	Asn	Ile	Ala	Val	Asp	Lys	Ala	Asn	Leu	Glu	Ile	Met	Thr	Lys	Arg
					165					170					175	
25	Ser	Asn	Tyr	Thr	Pro	Ile	Thr	Asn								
				180												
30	<210> CO599															
	<211> 184															
	<212> PRT															
	<213> Homo sapiens															
35	Met	Gly	Asp	Thr	Arg	Pro	Arg	Phe	Leu	Glu	Gln	Val	Lys	His	Glu	Cys
					5					10					15	
	His	Phe	Phe	Asn	Gly	Thr	Glu	Arg	Val	Arg	Phe	Leu	Asp	Arg	Tyr	Phe
				20					25					30		
40	Tyr	His	Gln	Glu	Glu	Tyr	Val	Arg	Phe	Asp	Ser	Asp	Val	Gly	Glu	Tyr
			35					40					45			
	Arg	Ala	Val	Thr	Glu	Leu	Gly	Arg	Pro	Asp	Ala	Glu	Tyr	Trp	Asn	Ser
45		50					55					60				
	Gln	Lys	Asp	Leu	Leu	Glu	Gln	Lys	Arg	Ala	Ala	Val	Asp	Thr	Tyr	Cys
	65					70					75					80
	Arg	His	Asn	Tyr	Gly	Val	Gly	Glu	Ser	Phe	Thr	Val	Gln	Arg	Arg	Gly
50					85					90					95	
	Gly	Gly	Gly	Ile	Lys	Glu	Glu	His	Val	Ile	Ile	Gln	Ala	Glu	Phe	Tyr
				100					105					110		
55	Leu	Asn	Pro	Asp	Gln	Ser	Gly	Glu	Phe	Met	Phe	Asp	Phe	Asp	Gly	Asp
			115					120					125			
	Glu	Ile	Phe	His	Val	Asp	Met	Ala	Lys	Lys	Glu	Thr	Val	Trp	Arg	Leu
	130															

65

Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala  
 145 150 155 160  
 5 Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser  
 165 170 175  
 Asn Tyr Thr Pro Ile Thr Asn  
 180  
 10  
 <210> CO594  
 <211> 187  
 <212> PRT  
 15 <213> Homo sapiens  
 Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
 5 10 15  
 20 His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
 20 25 30  
 Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
 35 40 45  
 25 Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
 50 55 60  
 Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
 30 65 70 75 80  
 Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Val  
 85 90 95  
 35 Tyr Pro Glu Val Thr Val Ile Lys Glu Glu His Val Ile Ile Gln Ala  
 100 105 110  
 Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe  
 115 120 125  
 40 Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val  
 130 135 140  
 Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly  
 45 145 150 155 160  
 Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr  
 165 170 175  
 50 Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn  
 180 185  
 55  
 <210> CO592  
 <211> 188  
 <212> PRT  
 <213> Homo sapiens  
 Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys

66

				5					10				15			
	His	Phe	Phe	Asn	Gly	Thr	Glu	Arg	Val	Arg	Phe	Leu	Asp	Arg	Tyr	Phe
				20					25				30			
5	Tyr	His	Gln	Glu	Glu	Tyr	Val	Arg	Phe	Asp	Ser	Asp	Val	Gly	Glu	Tyr
			35					40				45				
	Arg	Ala	Val	Thr	Glu	Leu	Gly	Arg	Pro	Asp	Ala	Glu	Tyr	Trp	Asn	Ser
10		50					55					60				
	Gln	Lys	Asp	Leu	Leu	Glu	Gln	Lys	Arg	Ala	Ala	Val	Asp	Thr	Tyr	Cys
	65					70				75					80	
15	Arg	His	Asn	Tyr	Gly	Val	Gly	Glu	Ser	Phe	Thr	Val	Gln	Arg	Arg	Phe
					85					90				95		
	Asp	Ala	Pro	Ser	Pro	Leu	Pro	Ile	Lys	Glu	Glu	His	Val	Ile	Ile	Gln
				100					105					110		
20	Ala	Glu	Phe	Tyr	Leu	Asn	Pro	Asp	Gln	Ser	Gly	Glu	Phe	Met	Phe	Asp
			115					120					125			
	Phe	Asp	Gly	Asp	Glu	Ile	Phe	His	Val	Asp	Met	Ala	Lys	Lys	Glu	Thr
25		130					135					140				
	Val	Trp	Arg	Leu	Glu	Glu	Phe	Gly	Arg	Phe	Ala	Ser	Phe	Glu	Ala	Gln
	145					150					155				160	
30	Gly	Ala	Leu	Ala	Asn	Ile	Ala	Val	Asp	Lys	Ala	Asn	Leu	Glu	Ile	Met
				165						170					175	
	Thr	Lys	Arg	Ser	Asn	Tyr	Thr	Pro	Ile	Thr	Asn					
				180					185							
35																
	<210>	CO591														
	<211>	187														
	<212>	PRT														
40	<213>	Homo sapiens														
	Met	Gly	Asp	Thr	Arg	Pro	Arg	Phe	Leu	Glu	Gln	Val	Lys	His	Glu	Cys
					5					10				15		
45	His	Phe	Phe	Asn	Gly	Thr	Glu	Arg	Val	Arg	Phe	Leu	Asp	Arg	Tyr	Phe
				20					25				30			
	Tyr	His	Gln	Glu	Glu	Tyr	Val	Arg	Phe	Asp	Ser	Asp	Val	Gly	Glu	Tyr
			35					40				45				
50	Arg	Ala	Val	Thr	Glu	Leu	Gly	Arg	Pro	Asp	Ala	Glu	Tyr	Trp	Asn	Ser
		50					55					60				
	Gln	Lys	Asp	Leu	Leu	Glu	Gln	Lys	Arg	Ala	Ala	Val	Asp	Thr	Tyr	Cys
55	65					70				75					80	
	Arg	His	Asn	Tyr	Gly	Val	Gly	Glu	Ser	Phe	Thr	Val	Gln	Arg	Arg	Ser
					85					90				95		

67

Gly Gly Ser Gly Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala  
 100 105 110

5 Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe  
 115 120 125

Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val  
 130 135 140

10 Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly  
 145 150 155 160

Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr  
 165 170 175

15 Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn  
 180 185

20 <210> CO590  
 <211> 187  
 <212> PRT  
 <213> Homo sapiens

25 Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
 5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
 20 25 30

30 Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
 35 40 45

35 Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
 50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
 65 70 75 80

40 Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Thr  
 85 90 95

Ser Gly Gly Gly Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala  
 100 105 110

45 Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe  
 115 120 125

Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val  
 130 135 140

50 Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly  
 145 150 155 160

55 Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr  
 165 170 175

Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn  
 180 185

```

5      <210> CO589
      <211> 190
      <212> PRT
      <213> Homo sapiens

Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys
          5          10          15

10    His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe
          20          25          30

15    Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr
          35          40          45

20    Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser
          50          55          60

25    Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
          65          70          75          80

30    Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly
          85          90          95

35    Ser Pro Gly Gly Gly Gly Pro Gly Ser Ile Lys Glu Glu His Val Ile
          100          105          110

40    Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met
          115          120          125

45    Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys
          130          135          140

50    Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu
          145          150          155          160

55    Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu
          165          170          175

60    Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn
          180          185

65    <210> CO588
      <211> 190
      <212> PRT
      <213> Homo sapiens

70    Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys
          5          10          15

75    His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe
          20          25          30

80    Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr
          35          40          45

85    Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser
          50          55          60

```

69

	50		55		60	
	Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys					
	65		70		75	80
5	Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly					
		85		90		95
10	Ser Pro Pro Gly Gly Pro Pro Gly Ser Ile Lys Glu Glu His Val Ile					
		100		105		110
	Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met					
		115		120		125
15	Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys					
		130		135		140
20	Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu					
			150		155	160
	Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu					
		165		170		175
25	Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn					
		180		185		
30	<210> CO587					
	<211> 194					
	<212> PRT					
	<213> Homo sapiens					
35	Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys					
		5		10		15
	His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe					
		20		25		30
40	Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr					
		35		40		45
	Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser					
		50		55		60
45	Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys					
		65		70		75
	Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly					
		85		90		95
50	Ser Pro Gly Gly Gly Gly Ser Gly Gly Gly Pro Gly Ser Ile Lys Glu					
		100		105		110
55	Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser					
		115		120		125
	Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp					
		130		135		140

70

Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe  
 145 150 155 160  
 5 Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys  
 165 170 175  
 Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr  
 180 185 190  
 10 Asn  
 15 <210> CO586  
 <211> 194  
 <212> PRT  
 <213> Homo sapiens  
 20 Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
 5 10 15  
 His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
 20 25 30  
 25 Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
 35 40 45  
 Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
 50 55 60  
 30 Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
 65 70 75 80  
 35 Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Thr  
 85 90 95  
 Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser Ile Lys Glu  
 100 105 110  
 40 Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser  
 115 120 125  
 Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp  
 130 135 140  
 45 Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe  
 145 150 155 160  
 50 Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys  
 165 170 175  
 Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr  
 180 185 190  
 55 Asn

&lt;210&gt; CO598

71

&lt;211&gt; 639

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

```

5  atgggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggcgctagc 60
   ggagggggcg gaagcggcgg aggggggggac acccgaccac gtttcttgga gcagggttaa 120
   catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
   caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
   ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
10 gccgcggttg acacctactg cagacacaac tacgggggttg gtgagagctt cacagtgcctg 360
   cgccgactcg gaggtgaaga tgacgaggca gatcaccatg tgatcatcca ggccgagttc 420
   tatctgaatc ctgaccaatc aggcgagttt atgtttgact ttgatggtga tgagattttc 480
   catgtggata tggcaaagaa ggagacggtc tggcggtctg aagaatttgg acgattttgc 540
   agctttgagg ctcaagggtgc attggccaac atagctgtgg acaaagccaa cctggaaatc 600
15 atgacaaagc gctccaacta tactccgata accaattaa 639

```

&lt;210&gt; CO597

&lt;211&gt; 639

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

```

20  atgggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggcgctagc 60
   ggagggggcg gaagcggcgg aggggggggac acccgaccac gtttcttgga gcagggttaa 120
   catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
25  caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
   ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
   gccgcggttg acacctactg cagacacaac tacgggggttg gtgagagctt cacagtgcag 360
   cgccgaggag gtggaagcgg cggaaatcaa gaagaacatg tgatcatcca ggccgagttc 420
   tatctgaatc ctgaccaatc aggcgagttt atgtttgact ttgatggtga tgagattttc 480
30  catgtggata tggcaaagaa ggagacggtc tggcggtctg aagaatttgg acgattttgc 540
   agctttgagg ctcaagggtgc attggccaac atagctgtgg acaaagccaa cctggaaatc 600
   atgacaaagc gctccaacta tactccgata accaattaa 639

```

&lt;210&gt; CO596

&lt;211&gt; 636

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

```

35  atgggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggcgctagc 60
   ggagggggcg gaagcggcgg aggggggggac acccgaccac gtttcttgga gcagggttaa 120
   catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
   caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
   ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
   gccgcggttg acacctactg cagacacaac tacgggggttg gtgagagctt cacagtgcag 360
45  cgccgaggag gtggaggcag catcaaagaa gaacatgtga tcatccaggc cgagttctat 420
   ctgaatcctg accaatcagg cgagtttatg tttgactttg atggtgatga gattttccat 480
   gtggatatgg caaagaagga gacggtctgg cggcttgaag aatttggacg atttgccagc 540
   tttgaggctc aaggtgcatt ggccaacata gctgtggaca aagccaacct ggaaatcatg 600
   acaaagcgct ccaactatac tccgatcacc aattaa 636
50

```

&lt;210&gt; CO595

&lt;211&gt; 633

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

```

55  atgggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggcgctagc 60
   ggagggggcg gaagcggcgg aggggggggac acccgaccac gtttcttgga gcagggttaa 120
   catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
   caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240

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72

5 ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300  
 gccgcggttg acacctactg cagacacaac tacgggggttg gtgagagctt cacagtgcag 360  
 cggcgaggag gtggaggcat caaagaagaa catgtgatca tccaggccga gttctatctg 420  
 aatcctgacc aatcaggcga gtttatgttt gactttgatg gtgatgagat tttccatgtg 480  
 gatatggcaa agaaggagac ggtctggcgg cttgaagaat ttggacgatt tggcagcttt 540  
 gaggtcaag gtgcattggc caacatagct gtggacaaag ccaacctgga aatcatgaca 600  
 aagcgctcca actatactcc gatcaccaat taa 633

10 <210> C0593  
 <211> 642  
 <212> DNA  
 <213> Homo sapiens

15 atggggggaca ccggtcgcag cttcacccctg gcctccagcg agaccggcgt gggcgctagc 60  
 ggagggggcg gaagcggcgg agggggggac acccgaccac gtttcttgga gcagggtaaa 120  
 catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180  
 caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240  
 ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300  
 gccgcggttg acacctactg cagacacaac tacgggggttg gtgagagctt cacagtgcag 360  
 20 cggcgagtct accctgaggt aactgtcatc aaagaagaac atgtgatcat ccaggccgag 420  
 ttctatctga atcctgacca atcaggcgag tttatgtttg actttgatgg tgatgagatt 480  
 ttccatgtgg atatggcaaa gaaggagacg gtctggcggc ttgaagaatt tggacgattt 540  
 gccagctttg aggctcaagg tgcattggcc aacatagctg tggacaaagc caacctggaa 600  
 atcatgacaa agcgctccaa ctatactccg atcaccaatt aa 642

25 <210> C0585  
 <211> 645  
 <212> DNA  
 <213> Homo sapiens

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 ggagggggcg gaagcggcgg agggggggac acccgaccac gtttcttgga gcagggtaaa 120  
 catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180  
 caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240  
 35 ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300  
 gccgcggttg acacctactg cagacacaac tacgggggttg gtgagagctt cacagtgcag 360  
 cggcgattcg acgcacctag cccactccca atcaaagaag aacatgtgat catccaggcc 420  
 gaggctctat tgaatcctga ccaatcaggc gagtttatgt ttgactttga tgggtgatgag 480  
 attttccatg tggatatggc aaagaaggag acggtctggc ggcttgaaga atttggacga 540  
 40 tttgccagct ttgaggctca aggtgcattg gccaacatag ctgtggacaa agccaacctg 600  
 gaaatcatga caaagcgctc caactatact ccgatcacca attaa 645

45 <210> C0584  
 <211> 642  
 <212> DNA  
 <213> Homo sapiens

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 catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180  
 caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240  
 ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300  
 gccgcggttg acacctactg cagacacaac tacgggggttg gtgagagctt cacagtgcag 360  
 cggcggaagtg gcggtagtgg cggtagtatc aaagaagaac atgtgatcat ccaggccgag 420  
 55 ttctatctga atcctgacca atcaggcgag tttatgtttg actttgatgg tgatgagatt 480  
 ttccatgtgg atatggcaaa gaaggagacg gtctggcggc ttgaagaatt tggacgattt 540  
 gccagctttg aggctcaagg tgcattggcc aacatagctg tggacaaagc caacctggaa 600  
 atcatgacaa agcgctccaa ctatactccg atcaccaatt aa 642

&lt;210&gt; C0583

&lt;211&gt; 642

&lt;212&gt; DNA

5 &lt;213&gt; Homo sapiens

atggggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggcgctagc 60  
 ggagggggcg gaagcggcgg aggggggggac acccgaccac gtttcttgga gcagggttaa 120  
 catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180  
 10 caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240  
 ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300  
 gccgcggttg acacctactg cagacacaac tacgggggttg gtgagagctt cacagtgcag 360  
 cggcgaacta gtggtggcgg tggcagcatc aaagaagaac atgtgatcat ccaggccgag 420  
 ttctatctga atcctgacca atcaggcgag tttatgtttg actttgatgg tgatgagatt 480  
 15 ttccatgtgg atatggcaaa gaaggagacg gtctggcggc ttgaagaatt tggacgattt 540  
 gccagctttg aggctcaagg tgcattggcc aacatagctg tggacaaagc caacctggaa 600  
 atcatgacaa agcgtccaa ctatactccg atcaccaatt aa 642

&lt;210&gt; C0582

20 &lt;211&gt; 651

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

atggggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggcgctagc 60  
 25 ggagggggcg gaagcggcgg aggggggggac acccgaccac gtttcttgga gcagggttaa 120  
 catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180  
 caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240  
 ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300  
 gccgcggttg acacctactg cagacacaac tacgggggttg gtgagagctt cacagtgcag 360  
 30 cggcgaggct ctcttgagg tggaggtcct ggatctatca aagaagaaca tgtgatcatc 420  
 caggccgagt tctatctgaa tctgaccaa tcaggcgagt ttatgtttga ctttgatggg 480  
 gatgagattt tccatgtgga tatggcaaag aaggagacgg tctggcggct tgaagaattt 540  
 ggacgatttg ccagctttga ggctcaagg tgcattggcca acatagctgt ggacaaagcc 600  
 aacctggaaa tcatgacaaa gcgctccaac tatactccga tcaccaatta a 651  
 35

&lt;210&gt; C0581

&lt;211&gt; 651

&lt;212&gt; DNA

40 &lt;213&gt; Homo sapiens

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 ggagggggcg gaagcggcgg aggggggggac acccgaccac gtttcttgga gcagggttaa 120  
 catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180  
 45 caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240  
 ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300  
 gccgcggttg acacctactg cagacacaac tacgggggttg gtgagagctt cacagtgcag 360  
 cggcgaggct ctctcctgg tggaccacct ggatctatca aagaagaaca tgtgatcatc 420  
 caggccgagt tctatctgaa tctgaccaa tcaggcgagt ttatgtttga ctttgatggg 480  
 50 gatgagattt tccatgtgga tatggcaaag aaggagacgg tctggcggct tgaagaattt 540  
 ggacgatttg ccagctttga ggctcaagg tgcattggcca acatagctgt ggacaaagcc 600  
 aacctggaaa tcatgacaaa gcgctccaac tatactccga tcaccaatta a 651

55 &lt;210&gt; C0580

&lt;211&gt; 663

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

74

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 ggagggggcg gaagcggcgg aggggggggac acccgaccac gtttcttgga gcagggttaa 120  
 catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180  
 5 caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240  
 ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300  
 gccgcggttg acacctactg cagacacaac tacgggggttg gtgagagctt cacagtgcag 360  
 cggcgagggg gtccaggcgg tggcgggagc ggcgggtggt ctgggagtat caaagaagaa 420  
 catgtgatca tccaggccga gttctatctg aatcctgacc aatcaggcga gtttatgttt 480  
 10 gactttgatg gtgatgagat tttccatgtg gatatggcaa agaaggagac ggtctggcgg 540  
 cttgaagaat ttggacgatt tgccagcttt gaggtcgaag gtgcattggc caacatagct 600  
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 taa 663

15 <210> C0567  
 <211> 663  
 <212> DNA  
 <213> Homo sapiens

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 catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180  
 caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240  
 25 ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300  
 gccgcggttg acacctactg cagacacaac tacgggggttg gtgagagctt cacagtgcag 360  
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 catgtgatca tccaggccga gttctatctg aatcctgacc aatcaggcga gtttatgttt 480  
 gactttgatg gtgatgagat tttccatgtg gatatggcaa agaaggagac ggtctggcgg 540  
 cttgaagaat ttggacgatt tgccagcttt gaggtcgaag gtgcattggc caacatagct 600  
 30 gtggacaaaag ccaacctgga aatcatgaca aagcgctcca actatactcc gatcaccaat 660  
 taa 663

35 <210> C0598  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens

40 Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly  
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 Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg  
 20 25 30  
 45 Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
 35 40 45  
 Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
 50 55 60  
 Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
 65 70 75 80  
 55 Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
 85 90 95  
 Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
 100 105 110

75

Val Gly Glu Ser Phe Thr Val Leu Arg Arg Leu Gly Gly Glu Asp Asp  
 115 120 125

5 Glu Ala Asp His His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro  
 130 135 140

Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe  
 145 150 155 160

10 His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe  
 165 170 175

Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala  
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15 Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr  
 195 200 205

20 Pro Ile Thr Asn  
 210

<210> CO597  
 <211> 213  
 25 <212> PRT  
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Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly  
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30 Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg  
 20 25 30

35 Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
 35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
 50 55 60

40 Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
 65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
 85 90 95

45 Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
 100 105 110

50 Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Gly Gly Ser Gly Gly  
 115 120 125

Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro  
 130 135 140

55 Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe  
 145 150 155 160

His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe  
 165 170 175

76

Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala  
 180 185 190

5 Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr  
 195 200 205

Pro Ile Thr Asn  
 210

10

<210> CO596  
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 15 <213> Homo sapiens

Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly  
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20 Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg  
 20 25 30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
 35 40 45

25 Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
 50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
 30 65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
 85 90 95

35 Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
 100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Gly Gly Gly Ser Ile  
 115 120 125

40 Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp  
 130 135 140

Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe His  
 45 145 150 155 160

Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe Gly  
 165 170 175

50 Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala Val  
 180 185 190

Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro  
 195 200 205

55 Ile Thr Asn  
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77

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<210> C0595
<211> 211
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[illegible]

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50      <210> C0593
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55 Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly  
5 10 15  
Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg  
20 25 30

78

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
                   35                                  40                                  45

5 Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
           50                                  55                                  60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
   65                                  70                                  75                                  80

10 Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
                                   85                                  90                                  95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
                                   100                                  105                                  110

15 Val Gly Glu Ser Phe Thr Val Gln Arg Arg Val Tyr Pro Glu Val Thr  
                   115                                  120                                  125

Val Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn  
 20       130                                  135                                  140

Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile  
   145                                  150                                  155                                  160

25 Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu  
                                   165                                  170                                  175

Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile  
                                   180                                  185                                  190

30 Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr  
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Thr Pro Ile Thr Asn  
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<210> CO585  
 <211> 215  
 40 <212> PRT  
     <213> Homo sapiens

Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly  
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45 Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg  
                                   20                                  25                                  30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
 50       35                                  40                                  45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
   50                                  55                                  60

55 Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
   65                                  70                                  75                                  80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
                                   85                                  90                                  95

79

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
 100 105 110  
 5 Val Gly Glu Ser Phe Thr Val Gln Arg Arg Phe Asp Ala Pro Ser Pro  
 115 120 125  
 Leu Pro Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu  
 130 135 140  
 10 Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu  
 145 150 155 160  
 Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu  
 165 170 175  
 15 Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn  
 180 185 190  
 20 Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn  
 195 200 205  
 Tyr Thr Pro Ile Thr Asn  
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 <210> CO584  
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 30 <213> Homo sapiens  
 Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly  
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 35 Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Asp Thr Arg  
 20 25 30  
 Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
 35 40 45  
 40 Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
 50 55 60  
 Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
 65 70 75 80  
 45 Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
 85 90 95  
 50 Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
 100 105 110  
 Val Gly Glu Ser Phe Thr Val Gln Arg Arg Ser Gly Gly Ser Gly Gly  
 115 120 125  
 55 Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn  
 130 135 140  
 Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile



80

	145		150		155		160
	Phe His Val Asp	Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu					
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5	Phe Gly Arg Phe	Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile					
		180		185		190	
10	Ala Val Asp Lys	Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr					
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	Thr Pro Ile Thr Asn						
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25	Val Gly Ala Ser Gly	Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg					
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	Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly						
		35		40		45	
30	Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu						
		50		55		60	
	Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu						
		65		70		75	80
35	Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu						
		85		90		95	
40	Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly						
		100		105		110	
	Val Gly Glu Ser Phe Thr Val Gln Arg Arg Thr Ser Gly Gly Gly Gly						
		115		120		125	
45	Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn						
		130		135		140	
	Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile						
		145		150		155	160
50	Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu						
		165		170		175	
	Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile						
		180		185		190	
55	Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr						
		195		200		205	

81

Thr Pro Ile Thr Asn  
210

5 <210> CO582  
<211> 217  
<212> PRT  
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10 Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly  
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Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg  
20 25 30

15 Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
35 40 45

20 Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
65 70 75 80

25 Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
100 105 110

30 Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Ser Pro Gly Gly Gly  
115 120 125

Gly Pro Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe  
130 135 140

35 Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly  
145 150 155 160

40 Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg  
165 170 175

Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu  
180 185 190

45 Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg  
195 200 205

Ser Asn Tyr Thr Pro Ile Thr Asn  
210 215

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55 <210> CO581  
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<212> PRT  
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Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly  
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82

Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg  
                     20                    25                    30

5 Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
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Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
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10 Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
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15 Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
                     85                    90                    95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
                     100                    105                    110

20 Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Ser Pro Pro Gly Gly  
                     115                    120                    125

Pro Pro Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe  
                     130                    135                    140

25 Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly  
                     145                    150                    155                    160

30 Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg  
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Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu  
                     180                    185                    190

35 Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg  
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Ser Asn Tyr Thr Pro Ile Thr Asn  
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<210> C0580  
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 45 <213> Homo sapiens

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50 Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg  
                     20                    25                    30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
                     35                    40                    45

55 Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
                     50                    55                    60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu

83

	65		70						75				80			
	Leu	Gly	Arg	Pro	Asp	Ala	Glu	Tyr	Trp	Asn	Ser	Gln	Lys	Asp	Leu	Leu
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5	Glu	Gln	Lys	Arg	Ala	Ala	Val	Asp	Thr	Tyr	Cys	Arg	His	Asn	Tyr	Gly
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	Val	Gly	Glu	Ser	Phe	Thr	Val	Gln	Arg	Arg	Gly	Ser	Pro	Gly	Gly	Gly
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	145					150					155					160
	Asp	Phe	Asp	Gly	Asp	Glu	Ile	Phe	His	Val	Asp	Met	Ala	Lys	Lys	Glu
					165					170					175	
20	Thr	Val	Trp	Arg	Leu	Glu	Glu	Phe	Gly	Arg	Phe	Ala	Ser	Phe	Glu	Ala
				180					185					190		
	Gln	Gly	Ala	Leu	Ala	Asn	Ile	Ala	Val	Asp	Lys	Ala	Asn	Leu	Glu	Ile
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	Val	Gly	Ala	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Asp	Thr	Arg
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	Pro	Arg	Phe	Leu	Glu	Gln	Val	Lys	His	Glu	Cys	His	Phe	Phe	Asn	Gly
			35					40					45			
45	Thr	Glu	Arg	Val	Arg	Phe	Leu	Asp	Arg	Tyr	Phe	Tyr	His	Gln	Glu	Glu
		50					55					60				
	Tyr	Val	Arg	Phe	Asp	Ser	Asp	Val	Gly	Glu	Tyr	Arg	Ala	Val	Thr	Glu
	65					70					75				80	
50	Leu	Gly	Arg	Pro	Asp	Ala	Glu	Tyr	Trp	Asn	Ser	Gln	Lys	Asp	Leu	Leu
					85					90					95	
	Glu	Gln	Lys	Arg	Ala	Ala	Val	Asp	Thr	Tyr	Cys	Arg	His	Asn	Tyr	Gly
55				100					105					110		
	Val	Gly	Glu	Ser	Phe	Thr	Val	Gln	Arg	Arg	Thr	Ser	Gly	Gly	Gly	Gly
			115					120					125			

84

Ser Gly Gly Gly Gly Ser Ser Ser Ile Lys Glu Glu His Val Ile Ile  
 130 135 140

5 Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe  
 145 150 155 160

Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu  
 165 170 175

10 Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala  
 180 185 190

Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile  
 195 200 205

15 Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn  
 210 215 220

I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.Ck nucleotide sequence

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 gggggcggaa gcggcggagg gggagactcc gaaaggcatt tcgtgttcca gttcaagggc 180  
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50 I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.Ck amino acid sequence

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55 Gly Ser Thr Gly Asp Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro  
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85

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10	Leu	Arg	Phe	Asp	Ser	Asp	Val	Gly	Glu	Tyr	Arg	Ala	Val	Thr	Glu	Leu	
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86

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5	Asn Ile Phe Pro Pro Val Ile Asn Ile Thr Trp Leu Arg Asn Ser Lys				
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	His Ser Phe His Lys Leu Ser Tyr Leu Thr Phe Ile Pro Ser Asp Asp				
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15	Asp Ile Tyr Asp Cys Lys Val Glu His Trp Gly Leu Glu Glu Pro Val				
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	Leu Lys His Trp Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala				
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20	Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu				
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25	Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro				
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	Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn				
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30	Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr				
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	Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His				
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<210> CO 608 variation  
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88

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20 Glu Cys His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg  
35 40 45

25 Tyr Phe Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly  
50 55 60

Glu Tyr Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp  
65 70 75 80

30 Asn Ser Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr  
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Tyr Cys Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg  
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35 Arg Gly Gly Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr  
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Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp  
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Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu  
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89

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Glu Cys His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg  
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Tyr Phe Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly  
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Asn Ser Gln Arg Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr  
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Tyr Cys Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg  
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Arg Gly Gly Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr  
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35

Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp  
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Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu  
145 150 155 160

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Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala  
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91

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&lt;212&gt; DNA

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92

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 gtgactgtcc cctccagcac ctggcccagc gagaccgtca cctgcaacgt tgcccacccg 1620  
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15

&lt;210&gt; IAS MBP 90-101 CH1.H.CH2

&lt;211&gt; 2053

&lt;212&gt; DNA

20 &lt;213&gt; murine

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&lt;210&gt; IAS MBP 90-101 CH1.H

93

<211> 1707  
 <212> DNA  
 <213> murine

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35  
 <210> MBP 1-14 CK  
 <211> 1686  
 <212> DNA  
 <213> murine

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   agcggagggg gcggaagcgg cggagggggg gactccgaaa ggcatttcgt gttccagttc 180
   aagggcgagt gctacttcac caacgggacg cagcgcatat gatctgtgga cagatacatc 240
45 tacaaccggg aggagtacct gcgcttcgac agcgacgtgg gcgagtaccg cgcggtgacc 300
   gagctggggc ggccagaccc cgagtactac aataagcagt acctggagca aacgcggggc 360
   gagctggaca cgggtgtgcag acacaactac gaggggggtg agaccacac ctccctgcgg 420
   cggcttgaac agcccaatgt cgtcatctcc ctgtccagga cagaggccct caaccaccac 480
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   tcaaattcca cccagctac caatgaggct cctcaagcga ctgtgttccc caagtcccct 1080
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94

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 10 ggtacc 1686

&lt;210&gt; MBP 1-14 CH1.H.CH2

&lt;211&gt; 2059

15 &lt;212&gt; DNA

&lt;213&gt; murine

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 20 agcggagggg gcggaagcgg cggaggggga gactccgaaa ggcatttcgt gttccagttc 180  
 aagggcgagt gctacttcac caacgggacg cagcgcatac gatctgtgga cagatacatc 240  
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55 &lt;210&gt; MBP 90-101 CH1.H.CH2.CH3

&lt;211&gt; 2343

&lt;212&gt; DNA--

&lt;213&gt; murine

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	gggggcgga	gcggcggagg	gggagactcc	gaaaggcatt	tcgtgttcca	gttcaagggc	180
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	tgc						2343

45 <212> PRT--  
<213> murine

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20 25 30

Glu Arg His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn  
50 55 60



96

	Gly	Thr	Gln	Arg	Ile	Arg	Ser	Val	Asp	Arg	Tyr	Ile	Tyr	Asn	Arg	Glu	
	65					70					75					80	
5	Glu	Tyr	Leu	Arg	Phe	Asp	Ser	Asp	Val	Gly	Glu	Tyr	Arg	Ala	Val	Thr	
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40	Tyr	Gln	Ser	Pro	Gly	Asp	Ile	Gly	Gln	Tyr	Thr	His	Glu	Phe	Asp	Gly	
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45	Leu	Pro	Glu	Phe	Gly	Gln	Leu	Thr	Ser	Phe	Asp	Pro	Gln	Gly	Gly	Leu	
	305					310					315					320	
	Gln	Asn	Ile	Ala	Thr	Gly	Lys	Tyr	Thr	Leu	Gly	Ile	Leu	Thr	Lys	Arg	
50					325					330					335		
	Ser	Asn	Ser	Thr	Pro	Ala	Thr	Asn	Glu	Ala	Pro	Gln	Ala	Thr	Val	Phe	
				340					345					350			
55	Pro	Lys	Ser	Pro	Val	Leu	Leu	Gly	Gln	Pro	Asn	Thr	Leu	Ile	Cys	Phe	
			355					360					365				
	Val	Asp	Asn	Ile	Phe	Pro	Pro	Val	Ile	Asn	Ile	Thr	Trp	Leu	Arg	Asn	
		370					375					380					

[illegible]

98

&lt;210&gt; IAS MBP 1-14 CH1.H

&lt;211&gt; 677

&lt;212&gt; PRT--

&lt;213&gt; murine

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5 10 15

10 Gly Ser Thr Gly Asp Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro  
20 25 30

Pro Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Ser Glu Arg  
35 40 45

15 His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn Gly Thr  
50 55 60

Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile Tyr Asn Arg Glu Glu Tyr  
65 70 75 80

20 Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu Leu  
85 90 95

Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu Glu Gln Thr  
100 105 110

25 Arg Ala Glu Leu Asp Thr Val Cys Arg His Asn Tyr Glu Gly Val Glu  
115 120 125

30 Thr His Thr Ser Leu Arg Arg Leu Glu Gln Pro Asn Val Val Ile Ser  
130 135 140

Leu Ser Arg Thr Glu Ala Leu Asn His His Asn Thr Leu Val Cys Ser  
145 150 155 160

35 Val Thr Asp Phe Tyr Pro Ala Lys Ile Lys Val Arg Trp Phe Arg Asn  
165 170 175

Gly Gln Glu Glu Thr Val Gly Val Ser Ser Thr Gln Leu Ile Arg Asn  
180 185 190

Gly Asp Trp Thr Phe Gln Val Leu Val Met Leu Glu Met Thr Pro Arg  
195 200 205

45 Arg Gly Glu Val Tyr Thr Cys His Val Glu His Pro Ser Leu Lys Ser  
210 215 220

Pro Ile Thr Val Glu Trp Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly  
225 230 235 240

50 Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser Glu Asp  
245 250 255

Asp Ile Glu Ala Asp His Val Gly Val Tyr Gly Thr Thr Val Tyr Gln  
260 265 270

55 Ser Pro Gly Asp Ile Gly Gln Tyr Thr His Glu Phe Asp Gly Asp Glu  
275 280 285

99

	Trp	Phe	Tyr	Val	Asp	Leu	Asp	Lys	Lys	Glu	Thr	Ile	Trp	Met	Leu	Pro	
	290						295					300					
5	Glu	Phe	Gly	Gln	Leu	Thr	Ser	Phe	Asp	Pro	Gln	Gly	Gly	Leu	Gln	Asn	
	305					310					315					320	
	Ile	Ala	Thr	Gly	Lys	Tyr	Thr	Leu	Gly	Ile	Leu	Thr	Lys	Arg	Ser	Asn	
					325					330					335		
10	Ser	Thr	Pro	Ala	Thr	Asn	Glu	Ala	Pro	Gln	Ala	Thr	Val	Phe	Pro	Lys	
				340					345					350			
	Ser	Pro	Val	Leu	Leu	Gly	Gln	Pro	Asn	Thr	Leu	Ile	Cys	Phe	Val	Asp	
			355					360					365				
15	Asn	Ile	Phe	Pro	Pro	Val	Ile	Asn	Ile	Thr	Trp	Leu	Arg	Asn	Ser	Lys	
	370						375					380					
	Ser	Val	Thr	Asp	Gly	Val	Tyr	Glu	Thr	Ser	Phe	Leu	Val	Asn	Arg	Asp	
20	385					390					395					400	
	His	Ser	Phe	His	Lys	Leu	Ser	Tyr	Leu	Thr	Phe	Ile	Pro	Ser	Asp	Asp	
					405					410					415		
25	Asp	Ile	Tyr	Asp	Cys	Lys	Val	Glu	His	Trp	Gly	Leu	Glu	Glu	Pro	Val	
				420					425					430			
	Leu	Lys	His	Trp	Ala	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ala	
			435					440					445				
30	Lys	Thr	Thr	Pro	Pro	Ser	Val	Tyr	Pro	Leu	Ala	Pro	Gly	Cys	Gly	Asp	
	450						455					460					
	Thr	Thr	Gly	Ser	Ser	Val	Thr	Leu	Gly	Cys	Leu	Val	Lys	Gly	Tyr	Phe	
35	465					470					475					480	
	Pro	Glu	Ser	Val	Thr	Val	Thr	Trp	Asn	Ser	Gly	Ser	Leu	Ser	Ser	Ser	
					485					490					495		
40	Val	His	Thr	Phe	Pro	Ala	Leu	Leu	Gln	Ser	Gly	Leu	Tyr	Thr	Met	Ser	
				500					505					510			
	Ser	Ser	Val	Thr	Val	Pro	Ser	Ser	Thr	Trp	Pro	Ser	Gln	Thr	Val	Thr	
			515					520					525				
45	Cys	Ser	Val	Ala	His	Pro	Ala	Ser	Ser	Thr	Thr	Val	Asp	Lys	Lys	Leu	
	530						535					540					
	Glu	Pro	Ser	Gly	Pro	Ile	Ser	Thr	Ile	Asn	Pro	Cys	Pro	Pro	Cys	Lys	
50	545					550					555					560	
	Glu	Cys	His	Lys	Cys	Pro	Ala	Pro	Asn	Leu	Glu	Gly	Gly	Pro	Ser	Val	
					565					570					575		
55	Phe	Ile	Phe	Pro	Pro	Asn	Ile	Lys	Asp	Val	Leu	Met	Ile	Ser	Leu	Thr	
				580					585					590			
	Pro	Lys	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	Glu	Asp	Asp	Pro	Asp	
			595					600					605				

100

Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala Gln  
 610 615 620

5 Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Ile Arg Val Val Ser  
 625 630 635 640

Thr Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys  
 645 650 655

10 Cys Lys Val Asn Asn Lys Asp Leu Pro Ser Pro Ile Glu Arg Thr Ile  
 660 665 670

15 Ser Lys Ile Lys  
 675

20 <210> IAS MBP 90-101 CH1.H.CH2  
 <211> 563  
 <212> PRT--  
 <213> murine

25 Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro  
 5 10 15

Gly Ser Thr Gly Asp Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro  
 20 25 30

30 Pro Ala Ser Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Ser  
 35 40 45

Glu Arg His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn  
 50 55 60

35 Gly Thr Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile Tyr Asn Arg Glu  
 65 70 75 80

Glu Tyr Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr  
 85 90 95

40 Glu Leu Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu Glu  
 100 105 110

45 Gln Thr Arg Ala Glu Leu Asp Thr Val Cys Arg His Asn Tyr Glu Gly  
 115 120 125

Val Glu Thr His Thr Ser Leu Arg Arg Leu Glu Gln Pro Asn Val Val  
 130 135 140

50 Ile Ser Leu Ser Arg Thr Glu Ala Leu Asn His His Asn Thr Leu Val  
 145 150 155 160

Cys Ser Val Thr Asp Phe Tyr Pro Ala Lys Ile Lys Val Arg Trp Phe  
 165 170 175

55 Arg Asn Gly Gln Glu Glu Thr Val Gly Val Ser Ser Thr Gln Leu Ile  
 180 185 190

Arg Asn Gly Asp Trp Thr Phe Gln Val Leu Val Met Leu Glu Met Thr

101

	195		200		205
	Pro Arg Arg Gly Glu Val Tyr Thr Cys His Val Glu His Pro Ser Leu				
	210		215		220
5	Lys Ser Pro Ile Thr Val Glu Trp Thr Ser Gly Gly Gly Gly Ser Gly				
	225		230		235 240
10	Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser				
		245		250	255
	Glu Asp Asp Ile Glu Ala Asp His Val Gly Val Tyr Gly Thr Thr Val				
		260		265	270
15	Tyr Gln Ser Pro Gly Asp Ile Gly Gln Tyr Thr His Glu Phe Asp Gly				
		275		280	285
	Asp Glu Trp Phe Tyr Val Asp Leu Asp Lys Lys Glu Thr Ile Trp Met				
		290		295	300
20	Leu Pro Glu Phe Gly Gln Leu Thr Ser Leu Ser Phe Asp Pro Gln Gly				
			310		315 320
25	Gly Leu Gln Asn Ile Ala Thr Gly Lys Tyr Thr Leu Gly Ile Leu Thr				
		325		330	335
	Lys Arg Ser Asn Ser Thr Pro Ala Thr Asn Glu Ala Pro Gln Ala Thr				
		340		345	350
30	Val Phe Pro Lys Ser Pro Val Leu Leu Gly Gln Pro Asn Thr Leu Ile				
		355		360	365
	Cys Phe Val Asp Asn Ile Phe Pro Pro Val Ile Asn Ile Thr Trp Leu				
		370		375	380
35	Arg Asn Ser Lys Ser Val Thr Asp Gly Val Tyr Glu Thr Ser Phe Leu				
			390		395 400
40	Val Asn Arg Asp His Ser Phe His Lys Leu Ser Tyr Leu Thr Phe Ile				
		405		410	415
	Pro Ser Asp Asp Asp Ile Tyr Asp Cys Lys Val Glu His Trp Gly Leu				
		420		425	430
45	Glu Glu Pro Val Leu Lys His Trp Ala Ser Gly Gly Gly Gly Ser Gly				
		435		440	445
	Gly Gly Gly Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro				
		450		455	460
50	Gly Ser Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val				
			470		475 480
55	Lys Gly Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser				
		485		490	495
	Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu				
		500		505	510

102

Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser  
 515 520 525  
 5 Glu Thr Val Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val  
 530 535 540  
 Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys  
 545 550 555 560  
 10 Thr Val  
 15 <210> IAS MBP 90-101 CH1.H  
 <211> 556  
 <212> PRT  
 <213> murine  
 20 Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro  
 5 10 15  
 Gly Ser Thr Gly Asp Met Ala Ser Gln Lys Arg Pro Ser Gln Arg His  
 20 25 30  
 25 Gly Ser Lys Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Ser  
 35 40 45  
 Glu Arg His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn  
 50 55 60  
 30 Gly Thr Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile Tyr Asn Arg Glu  
 65 70 75 80  
 Glu Tyr Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr  
 85 90 95  
 35 Glu Leu Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu Glu  
 100 105 110  
 40 Gln Thr Arg Ala Glu Leu Asp Thr Val Cys Arg His Asn Tyr Glu Gly  
 115 120 125  
 Val Glu Thr His Thr Ser Leu Arg Arg Leu Glu Gln Pro Asn Val Val  
 130 135 140  
 45 Ile Ser Leu Ser Arg Thr Glu Ala Leu Asn His His Asn Thr Leu Val  
 145 150 155 160  
 Cys Ser Val Thr Asp Phe Tyr Pro Ala Lys Ile Lys Val Arg Trp Phe  
 165 170 175  
 50 Arg Asn Gly Gln Glu Glu Thr Val Gly Val Ser Ser Thr Gln Leu Ile  
 180 185 190  
 55 Arg Asn Gly Asp Trp Thr Phe Gln Val Leu Val Met Leu Glu Met Thr  
 195 200 205  
 Pro Arg Arg Gly Glu Val Tyr Thr Cys His Val Glu His Pro Ser Leu  
 210 215 220

## 103

	Lys	Ser	Pro	Ile	Thr	Val	Glu	Trp	Thr	Ser	Gly	Gly	Gly	Gly	Ser	Gly	
	225					230					235					240	
5	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ser	Ser	
					245					250					255		
	Glu	Asp	Asp	Ile	Glu	Ala	Asp	His	Val	Gly	Val	Tyr	Gly	Thr	Thr	Val	
				260					265					270			
10	Tyr	Gln	Ser	Pro	Gly	Asp	Ile	Gly	Gln	Tyr	Thr	His	Glu	Phe	Asp	Gly	
			275					280					285				
15	Asp	Glu	Trp	Phe	Tyr	Val	Asp	Leu	Asp	Lys	Lys	Glu	Thr	Ile	Trp	Met	
	290						295					300					
	Leu	Pro	Glu	Phe	Gly	Gln	Leu	Thr	Ser	Phe	Asp	Pro	Gln	Gly	Gly	Leu	
	305					310					315					320	
20	Gln	Asn	Ile	Ala	Thr	Gly	Lys	Tyr	Thr	Leu	Gly	Ile	Leu	Thr	Lys	Arg	
					325					330					335		
	Ser	Asn	Ser	Thr	Pro	Ala	Thr	Asn	Glu	Ala	Pro	Gln	Ala	Thr	Val	Phe	
				340					345					350			
25	Pro	Lys	Ser	Pro	Val	Leu	Leu	Gly	Gln	Pro	Asn	Thr	Leu	Ile	Cys	Phe	
			355					360					365				
30	Val	Asp	Asn	Ile	Phe	Pro	Pro	Val	Ile	Asn	Ile	Thr	Trp	Leu	Arg	Asn	
	370						375					380					
	Ser	Lys	Ser	Val	Thr	Asp	Gly	Val	Tyr	Glu	Thr	Ser	Phe	Leu	Val	Asn	
	385					390				395						400	
35	Arg	Asp	His	Ser	Phe	His	Lys	Leu	Ser	Tyr	Leu	Thr	Phe	Ile	Pro	Ser	
					405					410					415		
	Asp	Asp	Asp	Ile	Tyr	Asp	Cys	Lys	Val	Glu	His	Trp	Gly	Leu	Glu	Glu	
				420					425					430			
40	Pro	Val	Leu	Lys	His	Trp	Ala	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	
			435					440					445				
45	Gly	Ala	Asp	Ala	Ala	Pro	Thr	Val	Ser	Ile	Phe	Pro	Pro	Ser	Ser	Glu	
	450						455					460					
	Gln	Leu	Thr	Ser	Gly	Gly	Ala	Ser	Val	Val	Cys	Phe	Leu	Asn	Asn	Phe	
	465					470					475					480	
50	Tyr	Pro	Lys	Asp	Ile	Asn	Val	Lys	Trp	Lys	Ile	Asp	Gly	Ser	Glu	Arg	
				485					490						495		
	Gln	Asn	Gly	Val	Leu	Asn	Ser	Trp	Thr	Asp	Gln	Asp	Ser	Lys	Asp	Ser	
				500					505					510			
55	Thr	Tyr	Ser	Met	Ser	Ser	Thr	Leu	Thr	Leu	Thr	Lys	Asp	Glu	Tyr	Glu	
			515					520					525				
	Arg	His	Asn	Ser	Tyr	Thr	Cys	Glu	Ala	Thr	His	Lys	Thr	Ser	Thr	Ser	



[illegible]

105

	Ser	Glu	Asp	Asp	Ile	Glu	Ala	Asp	His	Val	Gly	Val	Tyr	Gly	Thr	Thr	
				260					265					270			
5	Val	Tyr	Gln	Ser	Pro	Gly	Asp	Ile	Gly	Gln	Tyr	Thr	His	Glu	Phe	Asp	
			275					280					285				
	Gly	Asp	Glu	Trp	Phe	Tyr	Val	Asp	Leu	Asp	Lys	Lys	Glu	Thr	Ile	Trp	
		290					295					300					
10	Met	Leu	Pro	Glu	Phe	Gly	Gln	Leu	Thr	Ser	Phe	Asp	Pro	Gln	Gly	Gly	
	305					310					315					320	
	Leu	Gln	Asn	Ile	Ala	Thr	Gly	Lys	Tyr	Thr	Leu	Gly	Ile	Leu	Thr	Lys	
					325					330					335		
15	Arg	Ser	Asn	Ser	Thr	Pro	Ala	Thr	Asn	Glu	Ala	Pro	Gln	Ala	Thr	Val	
				340					345					350			
20	Phe	Pro	Lys	Ser	Pro	Val	Leu	Leu	Gly	Gln	Pro	Asn	Thr	Leu	Ile	Cys	
			355					360					365				
	Phe	Val	Asp	Asn	Ile	Phe	Pro	Pro	Val	Ile	Asn	Ile	Thr	Trp	Leu	Arg	
		370					375					380					
25	Asn	Ser	Lys	Ser	Val	Thr	Asp	Gly	Val	Tyr	Glu	Thr	Ser	Phe	Leu	Val	
	385					390					395					400	
	Asn	Arg	Asp	His	Ser	Phe	His	Lys	Leu	Ser	Tyr	Leu	Thr	Phe	Ile	Pro	
				405						410					415		
30	Ser	Asp	Asp	Asp	Ile	Tyr	Asp	Cys	Lys	Val	Glu	His	Trp	Gly	Leu	Glu	
				420					425					430			
35	Glu	Pro	Val	Leu	Lys	His	Trp	Ala	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	
		435						440					445				
	Gly	Gly	Ala	Lys	Thr	Thr	Pro	Pro	Ser	Val	Tyr	Pro	Leu	Ala	Pro	Gly	
		450					455					460					
40	Ser	Ala	Ala	Gln	Thr	Asn	Ser	Met	Val	Thr	Leu	Gly	Cys	Leu	Val	Lys	
	465					470					475					480	
	Gly	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Thr	Trp	Asn	Ser	Gly	Ser	Leu	
					485					490					495		
45	Ser	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Asp	Leu	Tyr	
				500					505					510			
50	Thr	Leu	Ser	Ser	Ser	Val	Thr	Val	Pro	Ser	Ser	Thr	Trp	Pro	Ser	Glu	
			515					520					525				
	Thr	Val	Thr	Cys	Asn	Val	Ala	His	Pro	Ala	Ser	Ser	Thr	Lys	Val	Asp	
		530					535					540					
55	Lys	Lys	Ile	Val	Pro	Arg	Asp	Cys	Gly	Cys	Lys	Pro	Cys	Ile	Cys	Thr	
	545					550					555					560	
	Val	Pro	Glu	Val	Ser	Ser	Val	Phe	Ile	Phe	Pro	Pro	Lys	Pro	Lys	Asp	
					565					570					575		

106

Val Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp  
 580 585 590

5 Ile Ser Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp  
 595 600 605

Val Glu Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn  
 610 615 620

10 Ser Thr Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp  
 625 630 635 640

15 Leu Asn Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro  
 645 650 655

Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala  
 660 665 670

20 Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp  
 675 680 685

Lys Val Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile  
 690 695 700

25 Thr Val Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn  
 705 710 715 720

30 Thr Gln Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys  
 725 730 735

Leu Asn Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys  
 740 745 750

35 Ser Val Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu  
 755 760 765

Ser His Ser Pro Gly Lys  
 770

40

<210> IAS MBP 1-14 CH1.H.CH2  
 <211> 561  
 <212> PRT  
 45 <213> murine

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro  
 5 10 15

50 Gly Ser Thr Gly Asp Met Ala Ser Gln Lys Arg Pro Ser Gln Arg His  
 20 25 30

Gly Ser Lys Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Ser  
 35 40 45

55 Glu Arg His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn  
 50 55 60

Gly Thr Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile Tyr Asn Arg Glu

107

	65		70		75		80
	Glu Tyr Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr						
		85			90		95
5	Glu Leu Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu Glu			105		110	
		100					
10	Gln Thr Arg Ala Glu Leu Asp Thr Val Cys Arg His Asn Tyr Glu Gly			120		125	
		115					
	Val Glu Thr His Thr Ser Leu Arg Arg Leu Glu Gln Pro Asn Val Val			135		140	
		130					
15	Ile Ser Leu Ser Arg Thr Glu Ala Leu Asn His His Asn Thr Leu Val			150		155	160
	Cys Ser Val Thr Asp Phe Tyr Pro Ala Lys Ile Lys Val Arg Trp Phe			165		170	175
20							
	Arg Asn Gly Gln Glu Glu Thr Val Gly Val Ser Ser Thr Gln Leu Ile			180		185	190
25	Arg Asn Gly Asp Trp Thr Phe Gln Val Leu Val Met Leu Glu Met Thr			195		200	205
	Pro Arg Arg Gly Glu Val Tyr Thr Cys His Val Glu His Pro Ser Leu			210		215	220
30	Lys Ser Pro Ile Thr Val Glu Trp Thr Ser Gly Gly Gly Gly Ser Gly			225		230	235
	Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser			245		250	255
35							
	Glu Asp Asp Ile Glu Ala Asp His Val Gly Val Tyr Gly Thr Thr Val			260		265	270
40	Tyr Gln Ser Pro Gly Asp Ile Gly Gln Tyr Thr His Glu Phe Asp Gly			275		280	285
	Asp Glu Trp Phe Tyr Val Asp Leu Asp Lys Lys Glu Thr Ile Trp Met			290		295	300
45	Leu Pro Glu Phe Gly Gln Leu Thr Ser Phe Asp Pro Gln Gly Gly Leu			305		310	315
	Gln Asn Ile Ala Thr Gly Lys Tyr Thr Leu Gly Ile Leu Thr Lys Arg			325		330	335
50							
	Ser Asn Ser Thr Pro Ala Thr Asn Glu Ala Pro Gln Ala Thr Val Phe			340		345	350
55	Pro Lys Ser Pro Val Leu Leu Gly Gln Pro Asn Thr Leu Ile Cys Phe			355		360	365
	Val Asp Asn Ile Phe Pro Pro Val Ile Asn Ile Thr Trp Leu Arg Asn			370		375	380

108

Ser Lys Ser Val Thr Asp Gly Val Tyr Glu Thr Ser Phe Leu Val Asn  
 385 390 395 400  
 5 Arg Asp His Ser Phe His Lys Leu Ser Tyr Leu Thr Phe Ile Pro Ser  
 405 410 415  
 Asp Asp Asp Ile Tyr Asp Cys Lys Val Glu His Trp Gly Leu Glu Glu  
 420 425 430  
 10 Pro Val Leu Lys His Trp Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly  
 435 440 445  
 Gly Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser  
 450 455 460  
 15 Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly  
 465 470 475 480  
 Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser  
 485 490 495  
 20 Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr  
 500 505 510  
 25 Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr  
 515 520 525  
 Val Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys  
 530 535 540  
 30 Lys Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val  
 545 550 555 560  
 35 <210> IAS MBP 90-101 CH1.H.CH2.CH3  
 <211> 773  
 <212> PRT  
 <213> murine  
 40 Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro  
 5 10 15  
 Gly Ser Thr Gly Asp Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro  
 20 25 30  
 45 Pro Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Ser Glu Arg  
 35 40 45  
 50 His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn Gly Thr  
 50 55 60  
 Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile Tyr Asn Arg Glu Glu Tyr  
 65 70 75 80  
 55 Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu Leu  
 85 90 95  
 Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu Glu Gln Thr  
 100 105 110

109

	Arg	Ala	Glu	Leu	Asp	Thr	Thr	Cys	Arg	His	Asn	Tyr	Glu	Gly	Val	Glu	
			115					120					125				
5	Thr	His	Thr	Ser	Leu	Arg	Arg	Leu	Glu	Gln	Pro	Asn	Val	Val	Ile	Ser	
			130				135					140					
	Leu	Ser	Arg	Thr	Glu	Ala	Leu	Asn	His	His	Asn	Thr	Leu	Val	Cys	Ser	
			145			150					155					160	
10	Val	Thr	Asp	Phe	Tyr	Pro	Ala	Lys	Ile	Lys	Val	Arg	Trp	Phe	Arg	Asn	
				165						170					175		
	Gly	Gln	Glu	Glu	Thr	Val	Gly	Val	Ser	Ser	Thr	Gln	Leu	Ile	Arg	Asn	
15				180					185					190			
	Gly	Asp	Trp	Thr	Phe	Gln	Val	Leu	Val	Met	Leu	Glu	Met	Thr	Pro	Arg	
			195					200					205				
20	Arg	Gly	Glu	Val	Tyr	Thr	Cys	His	Val	Glu	His	Pro	Ser	Leu	Lys	Ser	
			210				215					220					
	Pro	Ile	Thr	Val	Glu	Trp	Thr	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	
			225			230					235					240	
25	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ser	Ser	Glu	Asp	
				245						250					255		
	Asp	Ile	Glu	Ala	Asp	His	Val	Gly	Val	Tyr	Gly	Thr	Thr	Val	Tyr	Gln	
30				260					265					270			
	Ser	Pro	Gly	Asp	Ile	Gly	Gln	Tyr	Thr	His	Glu	Phe	Asp	Gly	Asp	Glu	
			275					280					285				
35	Trp	Phe	Tyr	Val	Asp	Leu	Asp	Lys	Lys	Glu	Thr	Ile	Trp	Met	Leu	Pro	
			290				295					300					
	Glu	Phe	Gly	Gln	Leu	Thr	Ser	Phe	Asp	Pro	Gln	Gly	Gly	Leu	Gln	Asn	
			305			310					315					320	
40	Ile	Ala	Thr	Gly	Lys	Tyr	Thr	Leu	Gly	Ile	Leu	Thr	Lys	Arg	Ser	Asn	
				325						330					335		
	Ser	Thr	Pro	Ala	Thr	Asn	Glu	Ala	Pro	Gln	Ala	Thr	Val	Phe	Pro	Lys	
45				340					345					350			
	Ser	Pro	Val	Leu	Leu	Gly	Gln	Pro	Asn	Thr	Leu	Ile	Cys	Phe	Val	Asp	
			355					360					365				
50	Asn	Ile	Phe	Pro	Pro	Val	Ile	Asn	Ile	Thr	Trp	Leu	Arg	Asn	Ser	Lys	
			370				375					380					
	Ser	Val	Thr	Asp	Gly	Val	Tyr	Glu	Thr	Ser	Phe	Leu	Val	Asn	Arg	Asp	
			385			390					395					400	
55	His	Ser	Phe	His	Lys	Leu	Ser	Tyr	Leu	Thr	Phe	Ile	Pro	Ser	Asp	Asp	
				405						410					415		
	Asp	Ile	Tyr	Asp	Cys	Lys	Val	Glu	His	Trp	Gly	Leu	Glu	Glu	Pro	Val	

110

	420	425	430
	Leu Lys His Trp Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Leu		
	435	440	445
5	Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala		
	450	455	460
10	Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr		
	465	470	475
	Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser		
	485	490	495
15	Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu		
	500	505	510
	Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val		
	515	520	525
20	Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys		
	530	535	540
25	Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro		
	545	550	555
	Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu		
	565	570	575
30	Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp Ile Ser		
	580	585	590
	Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu		
	595	600	605
35	Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr		
	610	615	620
	Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn		
	625	630	635
40	Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro		
	645	650	655
45	Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln		
	660	665	670
	Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val		
	675	680	685
50	Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val		
	690	695	700
	Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln		
	705	710	715
55	Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn		
	725	730	735

111

Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val  
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5 Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu Ser His  
 755 760 765

Ser Pro Gly Lys  
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 <213> Murine

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45 <210> pCRC199  
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 <212> DNA  
 <213> Murine

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112

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 25 <212> DNA  
 <213> Murine

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<210> pCRC187

113

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 <212> DNA  
 <213> Murine

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114

&lt;210&gt; pCB223

&lt;211&gt; 1676

&lt;212&gt; DNA

5 &lt;213&gt; Murine

&lt;400&gt; 8

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&lt;210&gt; pCB212

&lt;211&gt; 773

&lt;212&gt; DNA

40 &lt;213&gt; Murine

&lt;400&gt; 9

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&lt;211&gt; 702

&lt;212&gt; DNA

<213> Murine

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<211> 1013

20 <213> Murine

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	cacaagctgt	cttatctcac	cttcacccct	tctgacgatg	atattttatga	ctgcaagggtg	600
35	gagcactggg	gcctggagga	gccgggtctg	aaacactggg	aacctgagat	tccagccccc	660
	atgtcagaag	gatccgcca	aacaacagcc	ccatcgggtc	atccactggc	ccctgtgtgt	720
	ggagatacaa	ctggctcctc	ggtgactcta	ggatgcctgg	tcaagggtta	tttccctgag	780
	ccagtgacct	tgacctggaa	ctctggatct	ctgtccagtg	gtgtgacacac	cttcccagct	840
	gtcctgcagt	ctgacctcta	caacctcagc	agctcagtg	gtgtaacctc	gagcacctgg	900
	cccagccagt	ccatcacctg	caatgtggcc	caccgcggca	gcagaccaa	gggtggacaag	960
	aaaattgagc	ccagagggcc	cacaatcaag	ccctgtgctg	cataggcggc	cgc	1013

<211> 207

<213> Murine

Met Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro Pro Gly Gly Gly  
5 10 15

50    Ser Gly Asp    Ser Glu Arg His    Phe Val    Phe Gln Phe Lys Gly Glu Cys  
                    20                          25                                 30

Tyr Phe Thr Asn Gly Thr Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile  
35 40 45

55 Tyr Asn Arg Glu Glu Tyr Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr  
50 55 60

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys

116

	65				70						75				80	
	Gln	Tyr	Leu	Glu	Gln	Thr	Arg	Ala	Glu	Leu	Asp	Thr	Val	Cys	Arg	His
					85					90					95	
5	Asn	Tyr	Glu	Gly	Val	Glu	Thr	His	Thr	Ser	Leu	Arg	Arg	Leu	Gly	Gly
				100					105					110		
10	Glu	Asp	Asp	Ile	Glu	Ala	Asp	His	Val	Gly	Val	Tyr	Gly	Thr	Thr	Val
		115						120					125			
	Tyr	Gln	Ser	Pro	Gly	Asp	Ile	Gly	Gln	Tyr	Thr	His	Glu	Phe	Asp	Gly
	130						135					140				
15	Asp	Glu	Trp	Phe	Tyr	Val	Asp	Leu	Asp	Lys	Lys	Glu	Thr	Ile	Trp	Met
	145					150					155					160
	Leu	Pro	Glu	Phe	Gly	Gln	Leu	Thr	Ser	Phe	Asp	Pro	Gln	Gly	Gly	Leu
				165						170					175	
20	Gln	Asn	Ile	Ala	Thr	Gly	Lys	Tyr	Thr	Leu	Gly	Ile	Leu	Thr	Lys	Arg
				180					185					190		
25	Ser	Asn	Ser	Thr	Pro	Ala	Thr	Asn	His	His	His	His	His	His	His	
		195						200						205		
	<210> pCRC201															
	<211> 201															
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	<213> Murine															
	<400> 13															
35	Met	Phe	Lys	Asn	Ile	Val	Thr	Pro	Arg	Thr	Pro	Pro	Pro	Gly	Gly	Gly
				5						10					15	
	Ser	Gly	Asp	Ser	Glu	Arg	His	Phe	Val	Phe	Gln	Phe	Lys	Gly	Glu	Cys
				20					25					30		
40	Tyr	Phe	Thr	Asn	Gly	Thr	Gln	Arg	Ile	Arg	Ser	Val	Asp	Arg	Tyr	Ile
			35					40					45			
	Tyr	Asn	Arg	Glu	Glu	Tyr	Leu	Arg	Phe	Asp	Ser	Asp	Val	Gly	Glu	Tyr
	50						55					60				
45	Arg	Ala	Val	Thr	Glu	Leu	Gly	Arg	Pro	Asp	Pro	Glu	Tyr	Tyr	Asn	Lys
	65					70					75					80
50	Gln	Tyr	Leu	Glu	Gln	Thr	Arg	Ala	Glu	Leu	Asp	Thr	Val	Cys	Arg	His
					85					90					95	
	Asn	Tyr	Glu	Gly	Val	Glu	Thr	His	Thr	Ser	Leu	Arg	Arg	Leu	Gly	Gly
				100					105					110		
55	Glu	Asp	Asp	Ile	Glu	Ala	Asp	His	Val	Gly	Val	Tyr	Gly	Thr	Thr	Val
		115						120					125			
	Tyr	Gln	Ser	Pro	Gly	Asp	Ile	Gly	Gln	Tyr	Thr	His	Glu	Phe	Asp	Gly
	130						135					140				

117

Asp Glu Trp Phe Tyr Val Asp Leu Asp Lys Lys Glu Thr Ile Trp Met  
 145 150 155 160

5 Leu Pro Glu Phe Gly Gln Leu Thr Ser Phe Asp Pro Gln Gly Gly Leu  
 165 170 175

Gln Asn Ile Ala Thr Gly Lys Tyr Thr Leu Gly Ile Leu Thr Lys Arg  
 180 185 190

10 Ser Asn Ser Thr Pro Ala Thr Asn  
 195 200

15 <210> pCRC199  
 <211> 218  
 <212> PRT  
 <213> Murine

20 <400> 14  
 Met Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro Pro Ala Ser Gly  
 5 10 15

25 Gly Gly Gly Ser Gly Gly Gly Gly Asp Ser Glu Arg His Phe Val Phe  
 20 25 30

Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn Gly Thr Gln Arg Ile Arg  
 35 40 45

30 Ser Val Asp Arg Tyr Ile Tyr Asn Arg Glu Glu Tyr Leu Arg Phe Asp  
 50 55 60

Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu Leu Gly Arg Pro Asp  
 65 70 75 80

35 Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu Glu Gln Thr Arg Ala Glu Leu  
 85 90 95

40 Asp Thr Val Cys Arg His Asn Tyr Glu Gly Val Glu Thr His Thr Ser  
 100 105 110

Leu Arg Arg Leu Thr Ser Gly Gly Gly Ser Glu Asp Asp Ile Glu  
 115 120 125

45 Ala Asp His Val Gly Val Tyr Gly Thr Thr Val Tyr Gln Ser Pro Gly  
 130 135 140

Asp Ile Gly Gln Tyr Thr His Glu Phe Asp Gly Asp Glu Trp Phe Tyr  
 145 150 155 160

50 Val Asp Leu Asp Lys Lys Glu Thr Ile Trp Met Leu Pro Glu Phe Gly  
 165 170 175

Gln Leu Thr Ser Phe Asp Pro Gln Gly Gly Leu Gln Asn Ile Ala Thr  
 180 185 190

55 Gly Lys Tyr Thr Leu Gly Ile Leu Thr Lys Arg Ser Asn Ser Thr Pro  
 195 200 205

118

Ala Thr Asn His His His His His His  
210 215

5 <210> pCRC197  
<211> 212  
<212> PRT  
<213> Murine

10 <400> 15  
Met Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro Pro Ala Ser Gly  
5 10 15

15 Gly Gly Gly Ser Gly Gly Gly Gly Asp Ser Glu Arg His Phe Val Phe  
20 25 30

Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn Gly Thr Gln Arg Ile Arg  
35 40 45

20 Ser Val Asp Arg Tyr Ile Tyr Asn Arg Glu Glu Tyr Leu Arg Phe Asp  
50 55 60

Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu Leu Gly Arg Pro Asp  
65 70 75 80

25 Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu Glu Gln Thr Arg Ala Glu Leu  
85 90 95

30 Asp Thr Val Cys Arg His Asn Tyr Glu Gly Val Glu Thr His Thr Ser  
100 105 110

Leu Arg Arg Leu Thr Ser Gly Gly Gly Gly Ser Glu Asp Asp Ile Glu  
115 120 125

35 Ala Asp His Val Gly Val Tyr Gly Thr Thr Val Tyr Gln Ser Pro Gly  
130 135 140

Asp Ile Gly Gln Tyr Thr His Glu Phe Asp Gly Asp Glu Trp Phe Tyr  
145 150 155 160

40 Val Asp Leu Asp Lys Lys Glu Thr Ile Trp Met Leu Pro Glu Phe Gly  
165 170 175

Gln Leu Thr Ser Phe Asp Pro Gln Gly Gly Leu Gln Asn Ile Ala Thr  
180 185 190

Gly Lys Tyr Thr Leu Gly Ile Leu Thr Lys Arg Ser Asn Ser Thr Pro  
195 200 205

50 Ala Thr Asn  
210

55 <210> pCRC188  
<211> 548  
<212> PRT  
<213> Murine

<400> 16

119

	Met	Ala	Leu	Gln	Ile	Pro	Ser	Leu	Leu	Leu	Ser	Ala	Ala	Val	Val	Val	
					5					10					15		
5	Leu	Met	Val	Leu	Ser	Ser	Pro	Gly	Thr	Glu	Gly	Ala	Ser	Gly	Gly	Gly	
				20					25					30			
	Gly	Ser	Gly	Gly	Gly	Gly	Asp	Ser	Glu	Arg	His	Phe	Val	Phe	Gln	Phe	
			35					40					45				
10	Lys	Gly	Glu	Cys	Tyr	Phe	Thr	Asn	Gly	Thr	Gln	Arg	Ile	Arg	Ser	Val	
		50					55					60					
	Asp	Arg	Tyr	Ile	Tyr	Asn	Arg	Glu	Glu	Tyr	Leu	Arg	Phe	Asp	Ser	Asp	
15		65				70					75				80		
	Val	Gly	Glu	Tyr	Arg	Ala	Val	Thr	Glu	Leu	Gly	Arg	Pro	Asp	Pro	Glu	
					85					90					95		
	Tyr	Tyr	Asn	Lys	Gln	Tyr	Leu	Glu	Gln	Thr	Arg	Ala	Glu	Leu	Asp	Thr	
20				100					105					110			
	Val	Cys	Arg	His	Asn	Tyr	Glu	Gly	Val	Glu	Thr	His	Thr	Ser	Leu	Arg	
			115					120					125				
25	Arg	Leu	Glu	Gln	Pro	Asn	Val	Val	Ile	Ser	Leu	Ser	Arg	Thr	Glu	Ala	
		130				135						140					
	Leu	Asn	His	His	Asn	Thr	Leu	Val	Cys	Ser	Val	Thr	Asp	Phe	Tyr	Pro	
30		145				150					155					160	
	Ala	Lys	Ile	Lys	Val	Arg	Trp	Phe	Arg	Asn	Gly	Gln	Glu	Glu	Thr	Val	
					165					170					175		
	Gly	Val	Ser	Ser	Thr	Gln	Leu	Ile	Arg	Asn	Gly	Asp	Trp	Thr	Phe	Gln	
35				180					185					190			
	Val	Leu	Val	Met	Leu	Glu	Met	Thr	Pro	Arg	Arg	Gly	Glu	Val	Tyr	Thr	
			195					200					205				
40	Cys	His	Val	Glu	His	Pro	Ser	Leu	Lys	Ser	Pro	Ile	Thr	Val	Glu	Trp	
		210					215					220					
	Thr	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	
45		225				230					235				240		
	Ser	Gly	Gly	Gly	Gly	Ser	Ser	Ser	Glu	Asp	Asp	Ile	Glu	Ala	Asp	His	
					245					250					255		
	Val	Gly	Val	Tyr	Gly	Thr	Thr	Val	Tyr	Gln	Ser	Pro	Gly	Asp	Ile	Gly	
50				260					265					270			
	Gln	Tyr	Thr	His	Glu	Phe	Asp	Gly	Asp	Glu	Trp	Phe	Tyr	Val	Asp	Leu	
		275						280					285				
55	Asp	Lys	Lys	Glu	Thr	Ile	Trp	Met	Leu	Pro	Glu	Phe	Gly	Gln	Leu	Thr	
		290					295					300					
	Ser	Phe	Asp	Pro	Gln	Gly	Gly	Leu	Gln	Asn	Ile	Ala	Thr	Gly	Lys	Tyr	
		305				310					315					320	



120

Thr Leu Gly Ile Leu Thr Lys Arg Ser Asn Ser Thr Pro Ala Thr Asn  
 325 330 335  
 5 Glu Ala Pro Gln Ala Thr Val Phe Pro Lys Ser Pro Val Leu Leu Gly  
 340 345 350  
 Gln Pro Asn Thr Leu Ile Cys Phe Val Asp Asn Ile Phe Pro Pro Val  
 355 360 365  
 10 Ile Asn Ile Thr Trp Leu Arg Asn Ser Lys Ser Val Thr Asp Gly Val  
 370 375 380  
 15 Tyr Glu Thr Ser Phe Leu Val Asn Arg Asp His Ser Phe His Lys Leu  
 385 390 395 400  
 Ser Tyr Leu Thr Phe Ile Pro Ser Asp Asp Asp Ile Tyr Asp Cys Lys  
 405 410 415  
 20 Val Glu His Trp Gly Leu Glu Glu Pro Val Leu Lys His Trp Ala Ser  
 420 425 430  
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Asp Ala Ala Pro Thr Val  
 435 440 445  
 25 Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly Gly Ala Ser  
 450 455 460  
 30 Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile Asn Val Lys  
 465 470 475 480  
 Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val Leu Asn Ser Trp  
 485 490 495  
 35 Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr Ser Met Ser Ser Thr Leu  
 500 505 510  
 Thr Leu Thr Lys Asp Glu Tyr Glu Arg His Asn Ser Tyr Thr Cys Glu  
 515 520 525  
 40 Ala Thr His Lys Thr Ser Thr Ser Pro Ile Val Lys Ser Phe Asn Arg  
 530 535 540  
 45 Asn Glu Cys  
 545  
 <210> pCRC187  
 <211> 253  
 50 <212> PRT  
 <213> Murine  
 <400> 17  
 55 Met Gly Lys Lys Gln Thr Ala Val Ala Phe Ala Leu Ala Leu Leu Ala  
 5 10 15  
 Leu Ser Met Thr Pro Ala Tyr Ala Phe Lys Asn Ile Val Thr Pro Arg  
 20 25 30

121

Thr Pro Pro Pro Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp  
 35 40 45  
 5 Ser Glu Arg His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr  
 50 55 60  
 Asn Gly Thr Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile Tyr Asn Arg  
 65 70 75 80  
 10 Glu Glu Tyr Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val  
 85 90 95  
 Thr Glu Leu Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu  
 100 105 110  
 15 Glu Gln Thr Arg Ala Glu Leu Asp Thr Val Cys Arg His Asn Tyr Glu  
 115 120 125  
 Gly Val Glu Thr His Thr Ser Leu Arg Arg Leu Gly Gly Gly Gly Ser  
 130 135 140  
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly  
 145 150 155 160  
 25 Gly Gly Gly Ser Glu Asp Asp Ile Glu Ala Asp His Val Gly Val Tyr  
 165 170 175  
 Gly Thr Thr Val Tyr Gln Ser Pro Gly Asp Ile Gly Gln Tyr Thr His  
 180 185 190  
 30 Glu Phe Asp Gly Asp Glu Trp Phe Tyr Val Asp Leu Asp Lys Lys Glu  
 195 200 205  
 Thr Ile Trp Met Leu Pro Glu Phe Gly Gln Leu Thr Ser Phe Asp Pro  
 210 215 220  
 Gln Gly Gly Leu Gln Asn Ile Ala Thr Gly Lys Tyr Thr Leu Gly Ile  
 225 230 235 240  
 40 Leu Thr Lys Arg Ser Asn Ser Thr Pro Ala Thr Asn  
 245 250  
 45 <210> pCB229  
 <211> 354  
 <212> PRT  
 <213> Murine  
 <400> 18  
 50 Met Ala Leu Gln Ile Pro Ser Leu Leu Leu Ser Ala Ala Val Val Val  
 5 10 15  
 Leu Met Val Leu Ser Ser Pro Gly Thr Glu Gly Phe Lys Asn Ile Val  
 20 25 30  
 55 Thr Pro Arg Thr Pro Pro Pro Ala Ser Gly Gly Gly Gly Ser Gly Gly  
 35 40 45  
 Gly Gly Asp Ser Glu Arg His Phe Val Phe Gln Phe Lys Gly Glu Cys

[illegible]

123

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5  <210> p/cb223
    <211> 552
    <212> PRT
    <213> Murine

    <400> 19
Met Ala Leu Gln Ile Pro Ser Leu Leu Leu Ser Ala Ala Val Val Val
      5              10              15

10  Leu Met Val Leu Ser Ser Pro Gly Thr Glu Gly Glu Asp Asp Ile Glu
      20              25              30

15  Ala Asp His Val Gly Val Tyr Gly Thr Thr Val Tyr Gln Ser Pro Gly
      35              40              45

    Asp Ile Gly Gln Tyr Thr His Glu Phe Asp Gly Asp Glu Trp Phe Tyr
      50              55              60

20  Val Asp Leu Asp Lys Lys Glu Thr Ile Trp Met Leu Pro Glu Phe Gly
      65              70              75              80

    Gln Leu Thr Ser Phe Asp Pro Gln Gly Gly Leu Gln Asn Ile Ala Thr
      85              90              95

25  Gly Lys Tyr Thr Leu Gly Ile Leu Thr Lys Arg Ser Asn Ser Thr Pro
      100             105             110

    Ala Thr Asn Glu Ala Pro Gln Ala Thr Val Phe Pro Lys Ser Pro Val
      115             120             125

30  Leu Leu Gly Gln Pro Asn Thr Leu Ile Cys Phe Val Asp Asn Ile Phe
      130             135             140

35  Pro Pro Val Ile Asn Ile Thr Trp Leu Arg Asn Ser Lys Ser Val Thr
      145             150             155             160

    Asp Gly Val Tyr Glu Thr Ser Phe Leu Val Asn Arg Asp His Ser Phe
      165             170             175

40  His Lys Leu Ser Tyr Leu Thr Phe Ile Pro Ser Asp Asp Asp Ile Tyr
      180             185             190

    Asp Cys Lys Val Glu His Trp Gly Leu Glu Glu Pro Val Leu Lys His
      195             200             205

45  Trp Glu Pro Glu Ile Pro Ala Pro Met Ser Glu Gly Ser Ala Lys Thr
      210             215             220

50  Thr Ala Pro Ser Val Tyr Pro Leu Ala Pro Val Cys Gly Asp Thr Thr
      225             230             235             240

    Gly Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu
      245             250             255

55  Pro Val Thr Leu Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His
      260             265             270

    Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser

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124

	275		280		285
	Val Thr Val Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile Thr Cys Asn				
	290		295		300
5	Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Glu Pro				
	305		310		315 320
10	Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro				
		325		330	335
	Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Ile Lys				
		340		345	350
15	Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val Thr Cys Val Val Val				
		355		360	365
	Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp Phe Val Asn				
		370		375	380
20	Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr				
			390		395 400
25	Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln His Gln Asp				
		405		410	415
	Trp Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Asp Leu				
		420		425	430
30	Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly Ser Val Arg				
		435		440	445
	Ala Pro Gln Val Tyr Val Leu Pro Pro Pro Glu Glu Glu Met Thr Lys				
		450		455	460
35	Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp Phe Met Pro Glu Asp				
			470		475 480
40	Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys				
		485		490	495
	Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser				
		500		505	510
45	Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser				
		515		520	525
	Cys Ser Val Val His Glu Gly Leu His Asn His His Thr Thr Lys Ser				
		530		535	540
50	Phe Ser Arg Thr Pro Gly Lys				
			550		
55	<210> pCB212				
	<211> 331				
	<212> PRT				
	<213> Murine				

125

&lt;400&gt; 20

Met Ala Leu Gln Ile Pro Ser Leu Leu Leu Ser Ala Ala Val Val Val  
                                   5                                  10                                  15

5 Leu Met Val Leu Ser Ser Pro Gly Thr Glu Gly Glu Asp Asp Ile Glu  
                                   20                                  25                                  30

Ala Asp His Val Gly Val Tyr Gly Thr Thr Val Tyr Gln Ser Pro Gly  
                                   35                                  40                                  45

10 Asp Ile Gly Gln Tyr Thr His Glu Phe Asp Gly Asp Glu Trp Phe Tyr  
                                   50                                  55                                  60

15 Val Asp Leu Asp Lys Lys Glu Thr Ile Trp Met Leu Pro Glu Phe Gly  
                                   65                                  70                                  75                                  80

Gln Leu Thr Ser Phe Asp Pro Gln Gly Gly Leu Gln Asn Ile Ala Thr  
                                   85                                  90                                  95

20 Gly Lys Tyr Thr Leu Gly Ile Leu Thr Lys Arg Ser Asn Ser Thr Pro  
                                   100                                  105                                  110

Ala Thr Asn Glu Ala Pro Gln Ala Thr Val Phe Pro Lys Ser Pro Val  
                                   115                                  120                                  125

25 Leu Leu Gly Gln Pro Asn Thr Leu Ile Cys Phe Val Asp Asn Ile Phe  
                                   130                                  135                                  140

30 Pro Pro Val Ile Asn Ile Thr Trp Leu Arg Asn Ser Lys Ser Val Thr  
                                   145                                  150                                  155                                  160

Asp Gly Val Tyr Glu Thr Ser Phe Leu Val Asn Arg Asp His Ser Phe  
                                   165                                  170                                  175

35 His Lys Leu Ser Tyr Leu Thr Phe Ile Pro Ser Asp Asp Asp Ile Tyr  
                                   180                                  185                                  190

Asp Cys Lys Val Glu His Trp Gly Leu Glu Glu Pro Val Leu Lys His  
                                   195                                  200                                  205

40 Trp Glu Pro Glu Ile Pro Ala Pro Met Ser Glu Gly Ser Ala Lys Thr  
                                   210                                  215                                  220

45 Thr Ala Pro Ser Val Tyr Pro Leu Ala Pro Val Cys Gly Asp Thr Thr  
                                   225                                  230                                  235                                  240

Gly Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu  
                                   245                                  250                                  255

50 Pro Val Thr Leu Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His  
                                   260                                  265                                  270

Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser  
                                   275                                  280                                  285

55 Val Thr Val Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile Thr Cys Asn  
                                   290                                  295                                  300

Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Glu Pro

[illegible]

127

<211> 560  
<212> PRT  
<213> Murine

5 <400> 22

Met Ala Leu Gln Ile Pro Ser Leu Leu Leu Ser Ala Ala Val Val Val  
5 10 15

Leu Met Val Leu Ser Ser Pro Gly Thr Glu Gly Phe Lys Asn Ile Val  
10                      20                      25                      30

Thr Pro Arg Thr Pro Pro Pro Ala Ser Gly Gly Gly Gly Ser Gly Gly  
35 40 45

15 Gly Gly Asp Ser Glu Arg His Phe Val Phe Gln Phe Lys Gly Glu Cys  
50 55 60

Tyr Phe Thr Asn Gly Thr Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile  
65 70 75 80

20 Tyr Asn Arg Glu Glu Tyr Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr  
85 90 95

25      Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys  
                        100                        105                        110

Gln Tyr Leu Glu Gln Thr Arg Ala Glu Leu Asp Thr Val Cys Arg His  
115 120 125

30 Asn Tyr Glu Gly Val Glu Thr His Thr Ser Leu Arg Arg Leu Glu Gln  
130 135 140

Pro	Asn	Val	Val	Ile	Ser	Leu	Ser	Arg	Thr	Glu	Ala	Leu	Asn	His	His
145					150					155					160

35 Asn Thr Leu Val Cys Ser Val Thr Asp Phe Tyr Pro Ala Lys Ile Lys  
165 170 175

40 Val Arg Trp Phe Arg Asn Gly Gln Glu Glu Thr Val Gly Val Ser Ser  
180 185 190

Thr Gln Leu Ile Arg Asn Gly Asp Trp Thr Phe Gln Val Leu Val Met  
195 200 205

45    Leu Glu Met Thr Pro Arg    Arg Gly Glu Val Tyr Thr Cys His Val Glu  
                210                      215                                 220

His Pro Ser Leu Lys Ser Pro Ile Thr Val Glu Trp Thr Ser Gly Gly  
225 230 235 240

50 Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly  
245 250 255

55 Gly Ser Ser Ser Glu Asp Asp Ile Glu Ala Asp His Val Gly Val Tyr  
260 265 270

Gly Thr Thr Val Tyr Gln Ser Pro Gly Asp Ile Gly Gln Tyr Thr His  
275 280 285



128

	Glu	Phe	Asp	Gly	Asp	Glu	Trp	Phe	Tyr	Val	Asp	Leu	Asp	Lys	Lys	Glu	
	290						295					300					
5	Thr	Ile	Trp	Met	Leu	Pro	Glu	Phe	Gly	Gln	Leu	Thr	Ser	Phe	Asp	Pro	
	305					310					315					320	
	Gln	Gly	Gly	Leu	Gln	Asn	Ile	Ala	Thr	Gly	Lys	Tyr	Thr	Leu	Gly	Ile	
					325					330					335		
10	Leu	Thr	Lys	Arg	Ser	Asn	Ser	Thr	Pro	Ala	Thr	Asn	Glu	Ala	Pro	Gln	
				340					345					350			
	Ala	Thr	Val	Phe	Pro	Lys	Ser	Pro	Val	Leu	Leu	Gly	Gln	Pro	Asn	Thr	
			355					360					365				
15	Leu	Ile	Cys	Phe	Val	Asp	Asn	Ile	Phe	Pro	Pro	Val	Ile	Asn	Ile	Thr	
	370						375					380					
20	Trp	Leu	Arg	Asn	Ser	Lys	Ser	Val	Thr	Asp	Gly	Val	Tyr	Glu	Thr	Ser	
	385					390					395					400	
	Phe	Leu	Val	Asn	Arg	Asp	His	Ser	Phe	His	Lys	Leu	Ser	Tyr	Leu	Thr	
				405						410					415		
25	Phe	Ile	Pro	Ser	Asp	Asp	Asp	Ile	Tyr	Asp	Cys	Lys	Val	Glu	His	Trp	
				420					425					430			
	Gly	Leu	Glu	Glu	Pro	Val	Leu	Lys	His	Trp	Ala	Ser	Gly	Gly	Gly	Gly	
		435						440					445				
30	Ser	Gly	Gly	Gly	Gly	Ala	Asp	Ala	Ala	Pro	Thr	Val	Ser	Ile	Phe	Pro	
	450						455					460					
35	Pro	Ser	Ser	Glu	Gln	Leu	Thr	Ser	Gly	Gly	Ala	Ser	Val	Val	Cys	Phe	
	465					470					475					480	
	Leu	Asn	Asn	Phe	Tyr	Pro	Lys	Asp	Ile	Asn	Val	Lys	Trp	Lys	Ile	Asp	
				485						490					495		
40	Gly	Ser	Glu	Arg	Gln	Asn	Gly	Val	Leu	Asn	Ser	Trp	Thr	Asp	Gln	Asp	
				500					505					510			
	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Met	Ser	Ser	Thr	Leu	Thr	Leu	Thr	Lys	
			515					520					525				
45	Asp	Glu	Tyr	Glu	Arg	His	Asn	Ser	Tyr	Thr	Cys	Glu	Ala	Thr	His	Lys	
	530						535					540					
50	Thr	Ser	Thr	Ser	Pro	Ile	Val	Lys	Ser	Phe	Asn	Arg	Asn	Glu	Cys		
	545					550					555						

WHAT IS CLAIMED IS:

- 1                   1.       A multimeric complex comprising a first recombinant single chain  
2 MHC class II molecule and a second recombinant single chain MHC class II molecule,  
3 wherein the first and the second single chain MHC class II molecule each comprise an  $\alpha 1$   
4 domain and a  $\beta 1$  domain linked via an amino acid linker and a multimerization domain,  
5 and wherein the first and the second single chain MHC class II molecule are linked via  
6 the multimerization domain to form a multimeric complex.
- 1                   2.       The multimeric complex of claim 1, wherein the first and the  
2 second single chain MHC class II molecules are each linked to an antigenic peptide via an  
3 amino acid linker.
- 1                   3.       The multimeric complex of claim 2, wherein the peptides are the  
2 same.
- 1                   4.       The multimeric complex of claim 2, wherein the peptides are from  
2 the same antigen.
- 1                   5.       The multimeric complex of claim 2, wherein the peptides are from  
2 different antigens.
- 1                   6.       The multimeric complex of claim 2, wherein the peptides are  
2 selected from the group consisting of MBP83-102Y83, PLP 40-60, PLP 89-106, PLP 95-  
3 117, and PLP 185-206.
- 1                   7.       The multimeric complex of claim 1, wherein the first and the  
2 second MHC class II molecules are from the same MHC class II allele.
- 1                   8.       The multimeric complex of claim 1, wherein the first and the  
2 second MHC class II molecules are from different MHC class II alleles.
- 1                   9.       The multimeric complex of claim 7 or 8, wherein the alleles are  
2 selected from the group consisting of DRB1\*1501 and DRB5\*0101.

- 1                   10.     The multimeric complex of claim 7 or 8, wherein the first and the  
2     second single chain MHC class II molecules are each linked to an antigenic peptide via an  
3     amino acid linker.
- 1                   11.     The multimeric complex of claim 10, wherein the peptides are the  
2     same.
- 1                   12.     The multimeric complex of claim 10, wherein the peptides are  
2     from the same antigen.
- 1                   13.     The multimeric complex of claim 10, wherein the peptides are  
2     from different antigens.
- 1                   14.     The multimeric complex of claim 10, wherein the peptides are  
2     selected from the group consisting of MBP83-102Y83, PLP 40-60, PLP 89-106, PLP 95-  
3     117, and PLP 185-206.
- 1                   15.     The multimeric complex of claim 1, wherein the first and second  
2     MHC class II molecules are from a human.
- 1                   16.     The multimeric complex of claim 1, wherein the multimerization  
2     domains are covalently linked.
- 1                   17.     The multimeric complex of claim 1, wherein the multimerization  
2     domains are non-covalently linked.
- 1                   18.     The multimeric complex of claim 1, wherein the multimerization  
2     domain is a leucine zipper domain.
- 1                   19.     The multimeric complex of claim 1, wherein the multimerization  
2     domain is an immunoglobulin domain.
- 1                   20.     The multimeric complex of claim 19, wherein the immunoglobulin  
2     domain is a light chain constant domain or a heavy chain constant domain.

1                   21.     The multimeric complex of claim 1, wherein the first or the second  
2 MHC class II molecule is selected from the group consisting of I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.C $\kappa$ ,  
3 I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.CH1.H, I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.CH1.H.CH2, and I-As  
4 MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.CH1.H.CH2.CH3.

1                   22.     The multimeric complex of claim 1, further comprising a third and  
2 a fourth MHC class II molecule, wherein the third and the fourth MHC class II molecule  
3 each comprise an  $\alpha$ 1 domain and a  $\beta$ 1 domain linked via an amino acid linker and a  
4 multimerization domain, and wherein the first, second, third and fourth single chain MHC  
5 class II molecules are linked via the multimerization domain to form a multimeric  
6 complex.

1                   23.     The multimeric complex of claim 1, wherein the first and the  
2 second MHC class II molecules comprise  $\beta$ 1  $\beta$ 2 domains and  $\alpha$ 1  $\alpha$ 2 domains linked via an  
3 amino acid linker.

1                   24.     The multimeric complex of claim 1 or 2, wherein the linker is  
2 selected from the group consisting of GG, GGGG, GGGGS, GGGSG, ASGGGS, GGGG,  
3 TSGGGGS, GGGGSSS, GSPGGGSGGGPGS, GSPPGGPPGS, GSPGGGGPGS,  
4 TSGGGGS, SGGSGGS, FDAPSPLP, and VYPEVTV.

1                   25.     The multimeric complex of claim 1 or 2, wherein the linker is from  
2 a CD4 molecule.

1                   26.     The multimeric complex of claim 25, wherein the linker is selected  
2 from the group consisting of RNGQEEKAGVVSTGLI, RNGQETKAGVVSTGLI,  
3 YNQQEEKAGGVSTGLI, FRNGQEEKAGVVSTGLI, FRNGQETKAGVVSTGLI,  
4 FYNQQEEKAGGVSTGLI, and LNGQEEKAGMVSTGLI.

1                   27.     The multimeric complex of claim 1, wherein the first or the second  
2 MHC class II molecule is selected from the group consisting of CO523, CO543, CO563,  
3 CO567, CO580, CO581, CO582, CO583, CO584, CO585, CO586, CO587, CO588,  
4 CO589, CO590, CO591, CO592, CO593, CO594, CO595, CO596, CO597, and CO608.

1                   28.     The multimeric complex of claim 1, wherein the first and the  
2     second MHC class II molecule are independently selected from the group consisting of  
3     CO523, CO543, CO563, CO567, CO580, CO581, CO582, CO583, CO584, CO585,  
4     CO586, CO587, CO588, CO589, CO590, CO591, CO592, CO593, CO594, CO595,  
5     CO596, CO597, and CO608.

1                   29.     The multimeric complex of claim 1, wherein the first MHC class II  
2     or the second MHC class II molecule is encoded by a nucleic acid that codons optimized  
3     for prokaryotic expression.

1                   30.     The multimeric complex of claim 29, wherein the prokaryote is *E.*  
2     *coli*.

1                   31.     The multimeric complex of claim 29, wherein the nucleic acid  
2     encoding the first or the second MHC class II molecule is selected from the group  
3     consisting of CO528-AC and CO608-AC.

1                   32.     A pharmaceutical composition comprising the multimeric complex  
2     of claim 2.

1                   33.     The pharmaceutical composition of claim 32, further comprising an  
2     adjuvant.

1                   34.     A method of treating autoimmune disease in a subject, the method  
2     comprising administering an immunogenically effective amount of a pharmaceutical  
3     composition comprising the multimeric complex of claim 2.

1                   35.     The method of claim 34, wherein the subject is a human.

1                   36.     A recombinant nucleic acid encoding a single chain MHC class II  
2     molecule comprising an  $\alpha$ 1 domain and a  $\beta$ 1 domain linked via an amino acid linker,  
3     wherein the linker is selected from the group consisting of GG, GGGG, GGGGS,  
4     GGGSG, ASGGGSGGG, TSGGGGSGGGSSS, GSPGGGGSGGGPGS,  
5     GSPPGGPPGS, GSPGGGGPGS, TSGGGGS, SGGSGGS, FDAPSPLP, and VYPEVTV.

1                   37.     The nucleic acid of claim 36, wherein the MHC class II molecule is  
2     selected from the group consisting of CO523, CO543, CO563, CO567, CO580, CO581,  
3     CO582, CO583, CO584, CO585, CO586, CO587, CO588, CO589, CO590, CO591,  
4     CO592, CO593, CO594, CO595, CO596, CO597, and CO608.

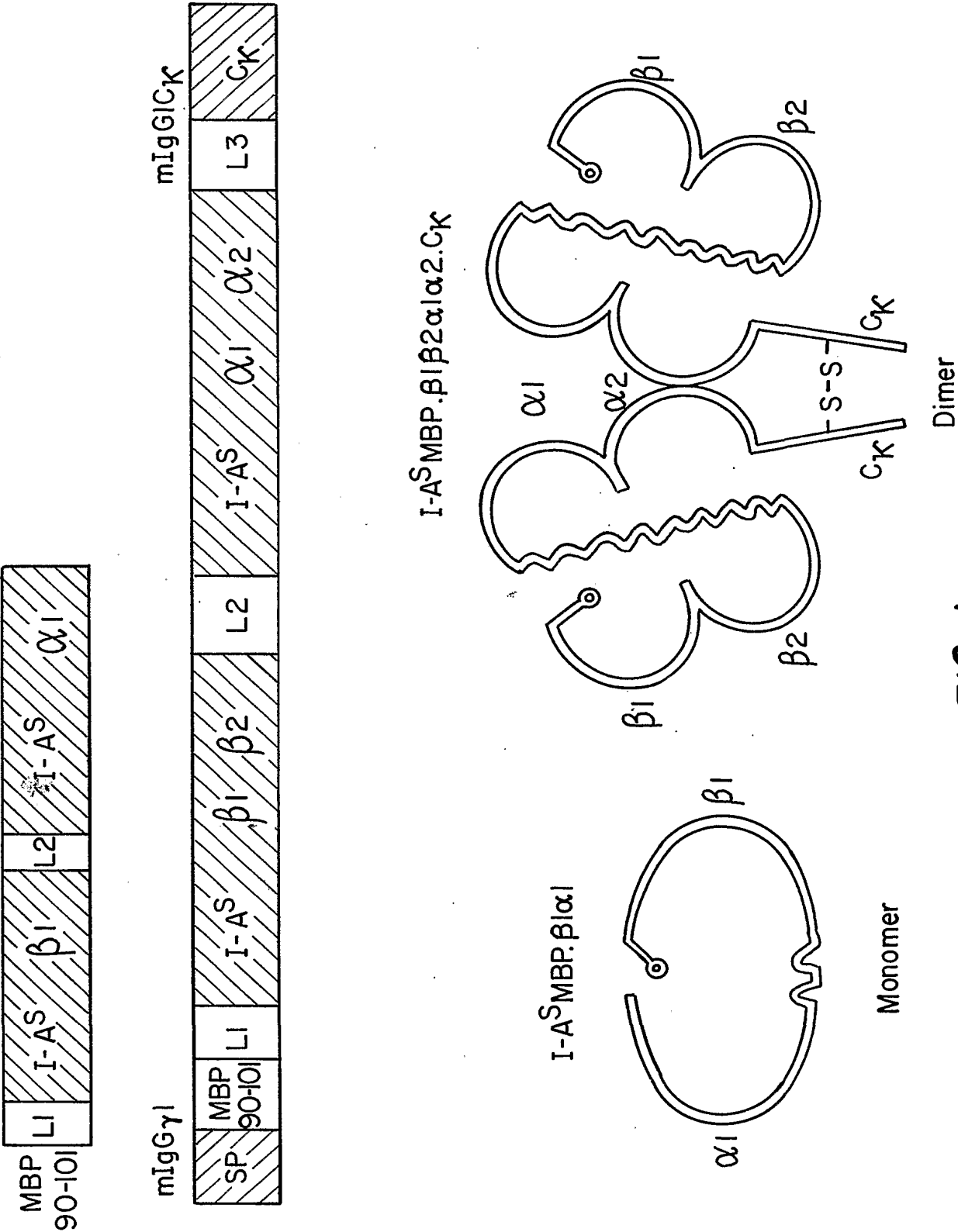
1                   38.     A recombinant nucleic acid encoding a single chain MHC class II  
2     molecule comprising an  $\alpha 1$  domain and a  $\beta 1$  domain linked via an amino acid linker,  
3     wherein the linker is wherein the linker is from a CD4 molecule.

1                   39.     The nucleic acid of claim 38, wherein the linker is selected from  
2     the group consisting of RNGQEEKAGVVSTGLI, RNGQETKAGVVSTGLI,  
3     YNQQEEKAGGVSTGLI, FRNGQEEKAGVVSTGLI, FRNGQETKAGVVSTGLI,  
4     FYNQQEEKAGGVSTGLI, and LNGQEEKAGMVSTGLI.

1                   40.     A recombinant nucleic acid encoding a single chain MHC class II  
2     molecule selected from the group consisting of CO528-AC and CO608-AC.

1                   41.     A recombinant nucleic acid encoding a single chain MHC class II  
2     molecule selected from the group consisting I-As MBP. $\beta 1\beta 2\alpha 1\alpha 2$ .C $\kappa$ , I-As  
3     MBP. $\beta 1\beta 2\alpha 1\alpha 2$ .CH1.H, I-As MBP. $\beta 1\beta 2\alpha 1\alpha 2$ .CH1.H.CH2, and I-As  
4     MBP. $\beta 1\beta 2\alpha 1\alpha 2$ .CH1.H.CH2.CH3.

1                   42.     A recombinant nucleic acid encoding a single chain MHC class II  
2     molecule selected from the group consisting CO523, CO543, CO563, CO567, CO580,  
3     CO581, CO582, CO583, CO584, CO585, CO586, CO587, CO588, CO589, CO590,  
4     CO591, CO592, CO593, CO594, CO595, CO596, CO597, and CO608.



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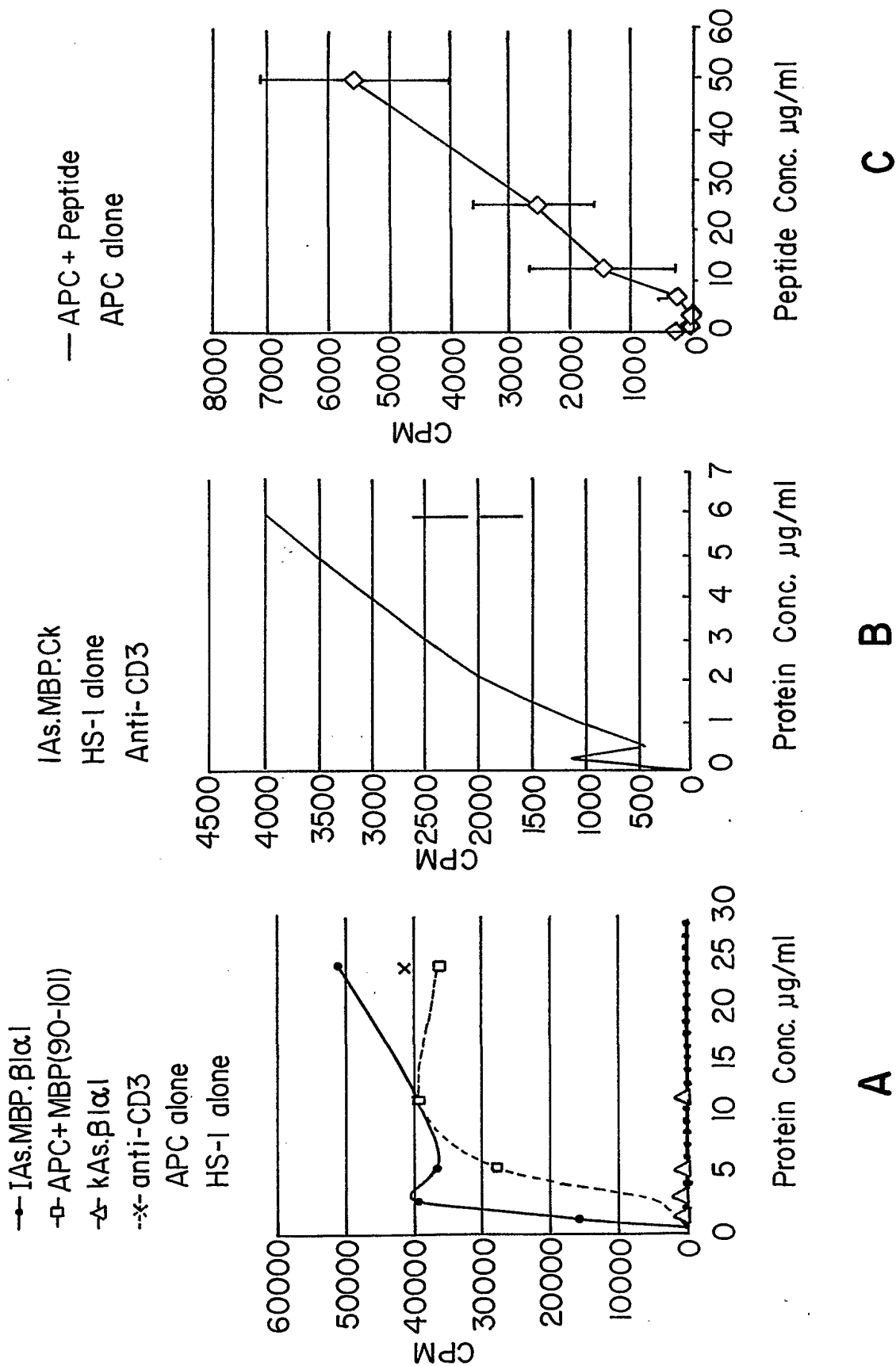


FIG. 2.



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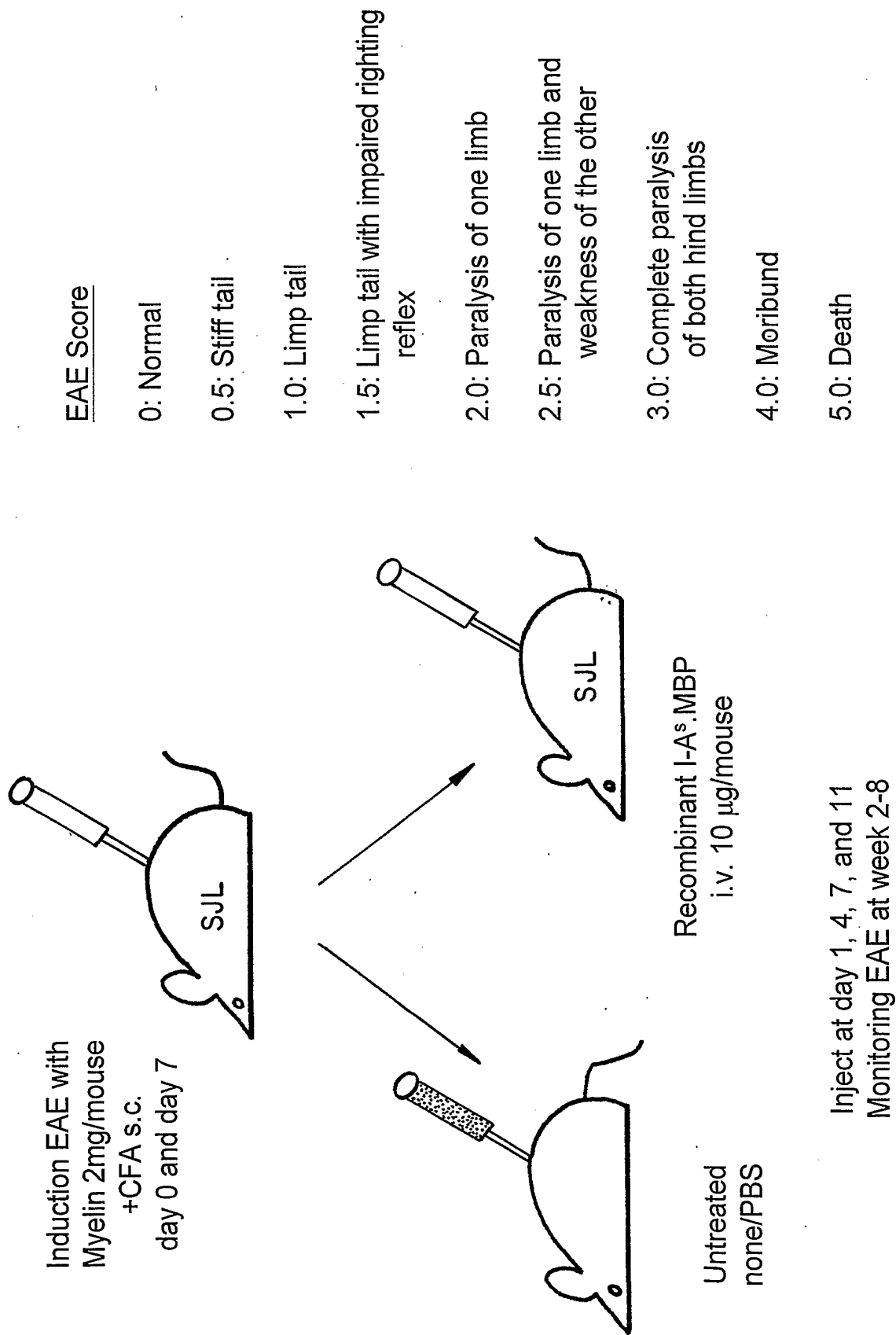
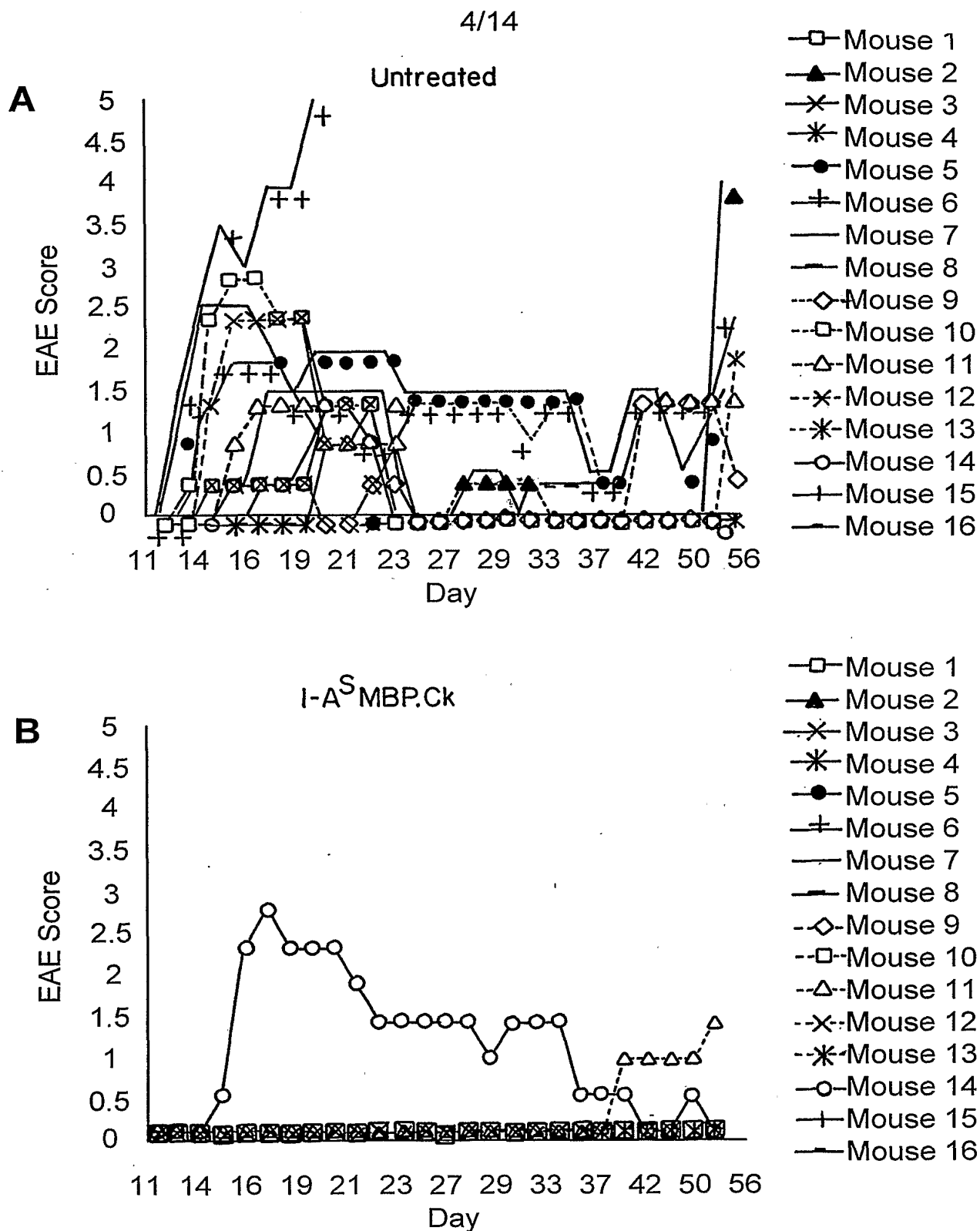
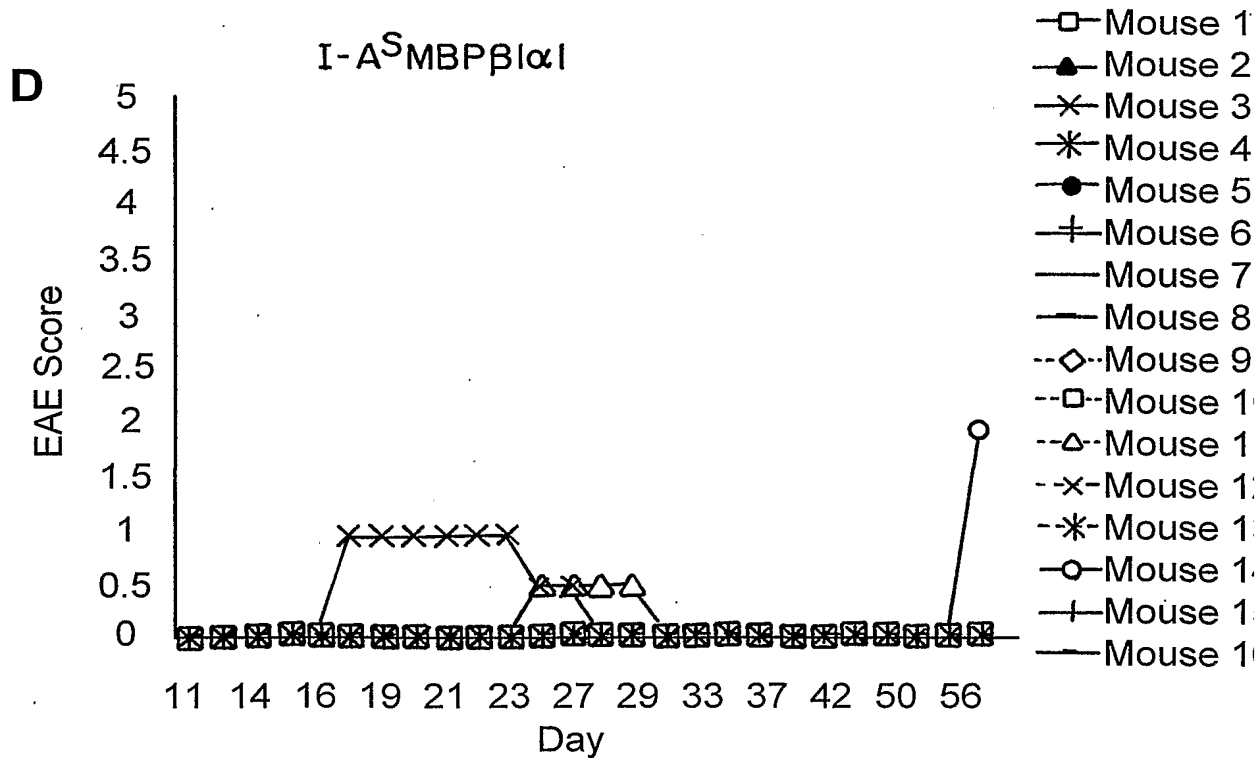
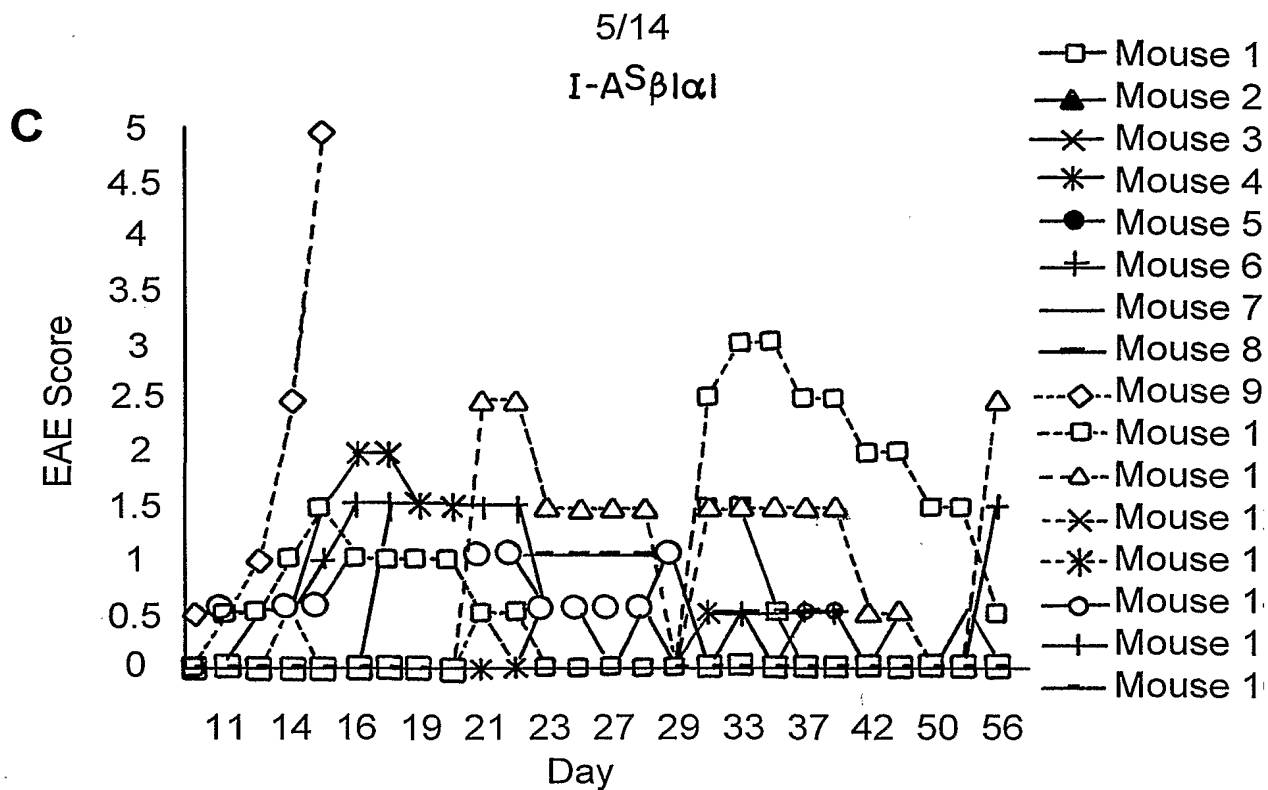


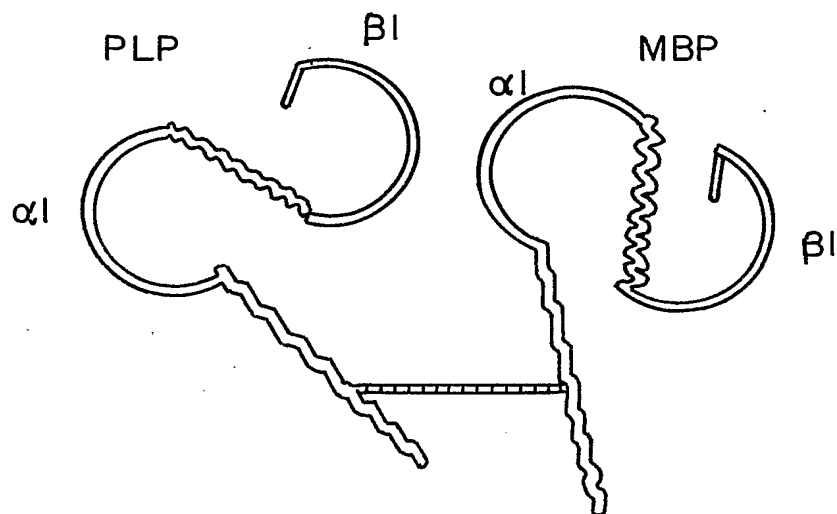
FIG. 3.

**FIG. 4.**

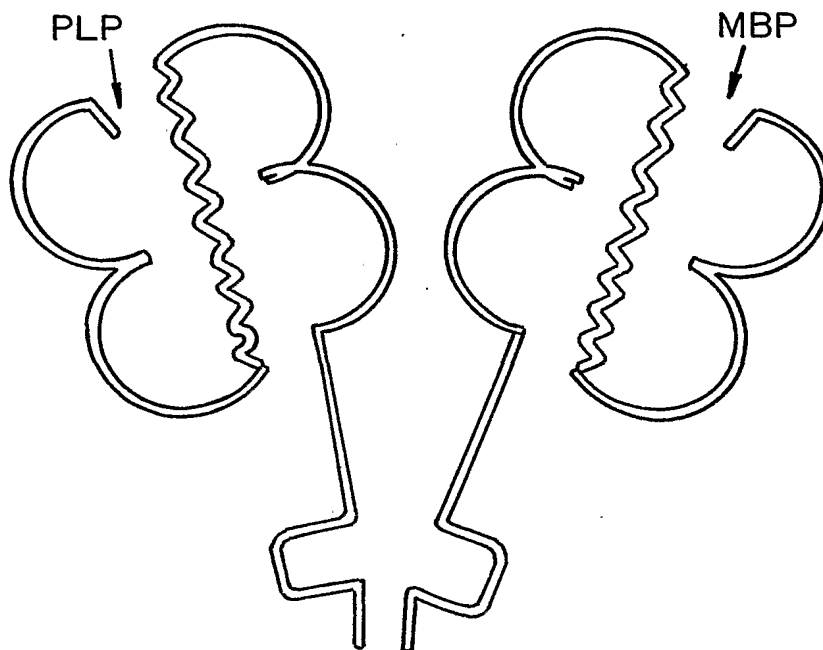


**FIG. 4.** (Continued)

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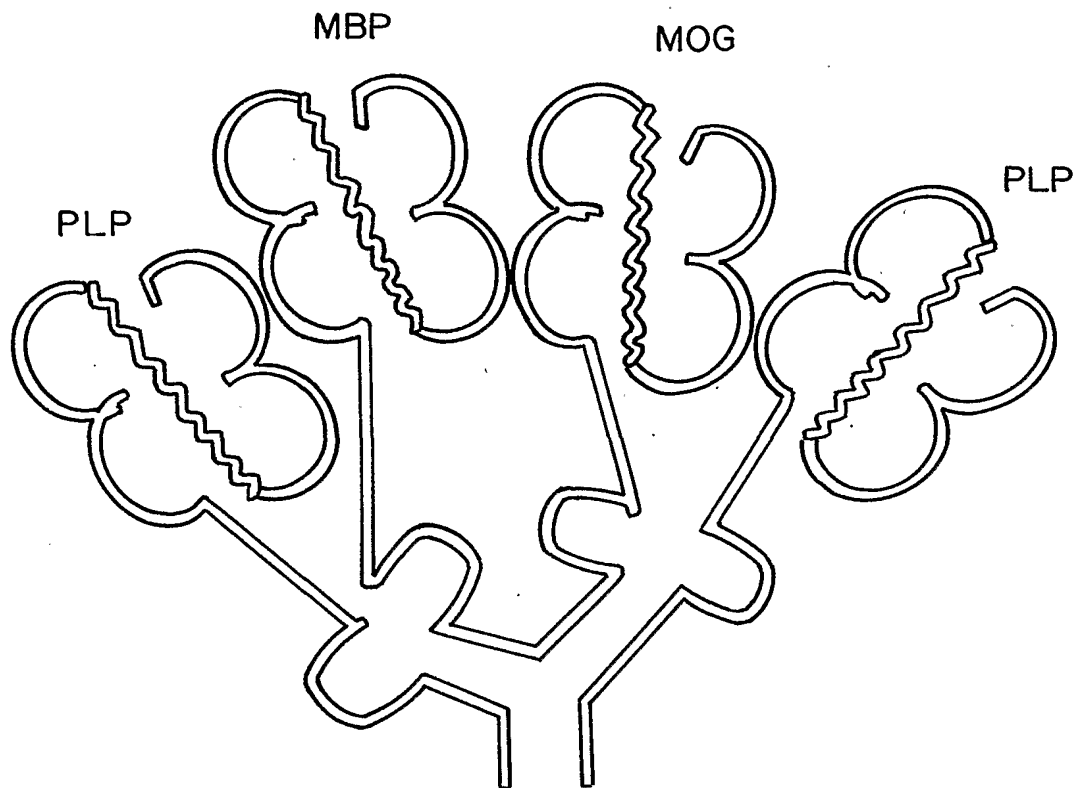


**FIG. 5.**



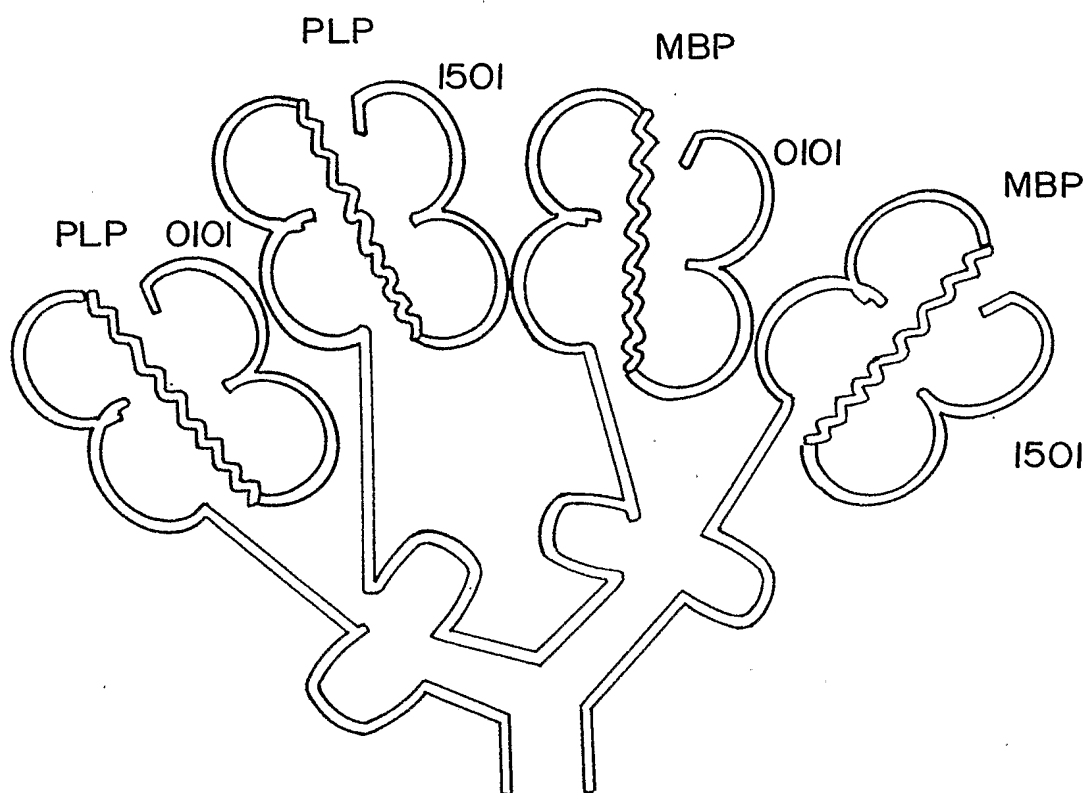
**FIG. 6.**

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**FIG. 7.**

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**FIG. 8.**

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EAE induction Day 0 and 7	Treatment Day 1, 4, 7 & 11 10ug/injection i.v.	Incidence of disease (%)
Myelin 2 mg/mouse in CFA s.c.	none	12/16 (75%)
Myelin 2 mg/mouse in CFA s.c.	Monomer	2/12 (16%)
Myelin 2 mg/mouse in CFA s.c.	Dimer	2/6 (33%)
Myelin 2 mg/mouse in CFA s.c.	Tetramer	3/14 (21%)
Myelin 2 mg/mouse in CFA s.c.	Monomer Pool	2/16 (12%)
Myelin 2 mg/mouse in CFA s.c.	Half Anergix	2/16 (12%)
Myelin 2 mg/mouse in CFA s.c.	I-As peptide	9/16 (56%)
Myelin 2 mg/mouse in CFA s.c.	Empty Half Anergix	10/16 (62%)

**FIG. 9.**

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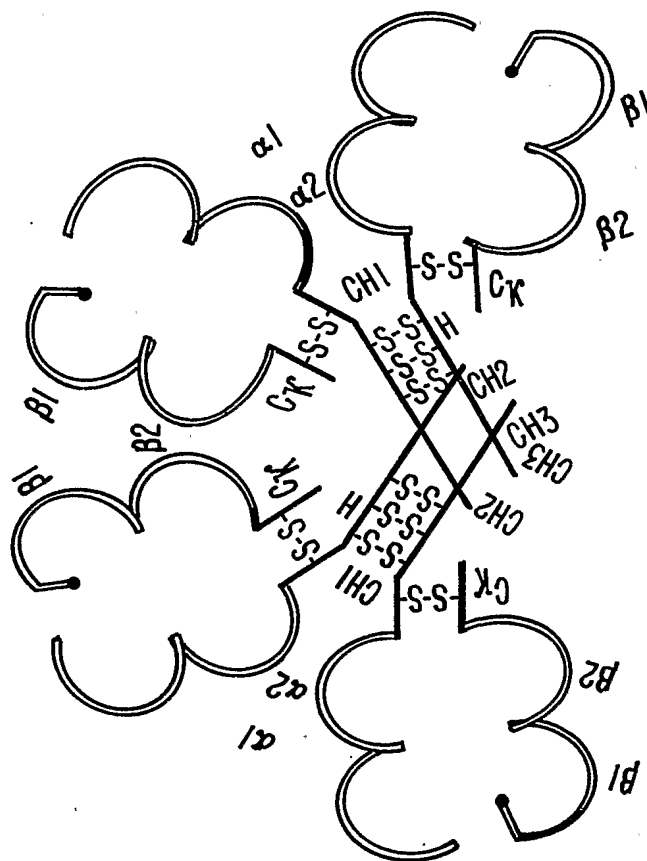
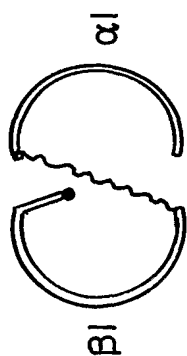
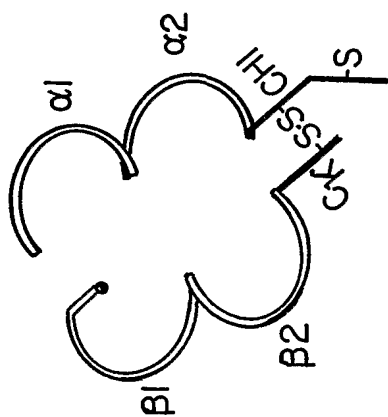
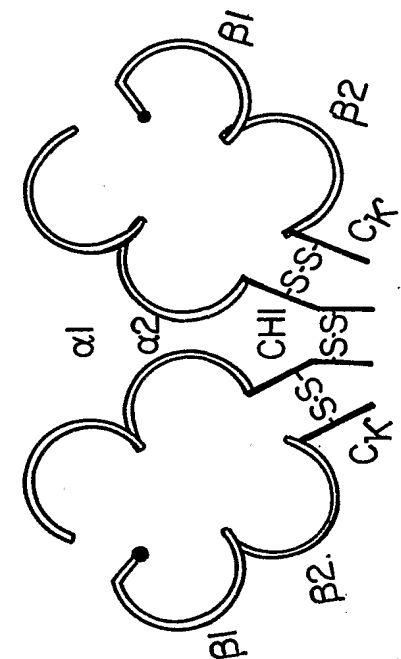


FIG. 10.



Native IAs beta 1.PRO	-----GDSEHFVFQFKG	13
mCO608.PRO	MGSEFKNIVTPRTPPPASGGSGGG-----HFVFQFKG	35
mCO608 A .pro	M-----FKNIVTPRTPPPASGGSGGG-----HFVFQFKG	31
mCO608 B.pro	M-----FKNIVTPRTPPPASGGSGGGG-----SERHFVFQFKG	35
pCRC197.pro	M-----FKNIVTPRTPPPASGGSGGGGDSERHFVFQFKG	36
pCRC199.pro	M-----FKNIVTPRTPPPASGGSGGGGDSERHFVFQFKG	36
Native IAs beta 1.PRO	KCYFTNGTQIRISVDRIYINREEYILRFSDVGEYRAVTEL	53
mCO608.PRO	KCYFTNGTQIRISVDRIYINREEYILRFSDVGEYRAVTEL	75
mCO608 A .pro	KCYFTNGTQIRISVDRIYINREEYILRFSDVGEYRAVTEL	71
mCO608 B.pro	KCYFTNGTQIRISVDRIYINREEYILRFSDVGEYRAVTEL	75
pCRC197.pro	KCYFTNGTQIRISVDRIYINREEYILRFSDVGEYRAVTEL	76
pCRC199.pro	KCYFTNGTQIRISVDRIYINREEYILRFSDVGEYRAVTEL	76
Native IAs beta 1.PRO	GRDPPEYYNKQYLEQTRAELDTVCRHNYEGVETHTSLR-	92
mCO608.PRO	GRDPPEYYNKQYLEQTRAELDTVCRHNYEGVETHTSLRRL	115
mCO608 A .pro	GRDPPEYYNKQYLEQTRAELDTVCRHNYEGVETHTSLRRL	111
mCO608 B.pro	GRDPPEYYNKQYLEQTRAELDTVCRHNYEGVETHTSLRRL	115
pCRC197.pro	GRDPPEYYNKQYLEQTRAELDTVCRHNYEGVETHTSLRRL	116
pCRC199.pro	GRDPPEYYNKQYLEQTRAELDTVCRHNYEGVETHTSLRRL	116
Native IAs beta 1.PRO	-----	92
mCO608.PRO	TSGGGSEDDIEADHVGVGTYQSPGDIGQYTHEFDGD	115
mCO608 A .pro	TSGGGSEDDIEADHVGVGTYQSPGDIGQYTHEFDGD	151
mCO608 B.pro	TSGGGSEDDIEADHVGVGTYQSPGDIGQYTHEFDGD	155
pCRC197.pro	TSGGGSEDDIEADHVGVGTYQSPGDIGQYTHEFDGD	156
pCRC199.pro	TSGGGSEDDIEADHVGVGTYQSPGDIGQYTHEFDGD	156

FIG. 11.

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Native IAs beta 1.PRO	-----	92
mCO608.PRO	EWFYVDLDKKETIWMLPEFGQLTSFDPQGGQLQNIATGKYT	195
mCO608 A .pro	EWFYVDLDKKETIWMLPEFGQLTSFDPQGGQLQNIATGKYT	191
mCO608 B.pro	EWFYVDLDKKETIWMLPEFGQLTSFDPQGGQLQNIATGKYT	195
pCRC197.pro	EWFYVDLDKKETIWMLPEFGQLTSFDPQGGQLQNIATGKYT	196
pCRC199.pro	EWFYVDLDKKETIWMLPEFGQLTSFDPQGGQLQNIATGKYT	196
Native IAs beta 1.PRO	-----L	93
mCO608.PRO	LGILTKRSNSTPATN.	211
mCO608 A .pro	LGILTKRSNSTPATN.	207
mCO608 B.pro	LGILTKRSNSTPATN.	211
pCRC197.pro	LGILTKRSNSTPATN.	212
pCRC199.pro	LGILTKRSNSTPATNHHHHHH.	218

FIG. 11. (CONTINUED)

Native IAs beta 1.PRO	-----GDSEHFVFQFKGECYFTN	19
CO561.pro	MGSEFKNIVTPRTPPPGGGS-----HFVFQFKGECYFTN	35
pCRC201.pro	M-----FKNIVTPRTPPPGGSGDSEHFVFQFKGECYFTN	36
pCRC203.pro	M-----FKNIVTPRTPPPGGSGDSEHFVFQFKGECYFTN	36
Native IAs beta 1.PRO	GTQRIRSVDRYIYNREEYLRFDSDVGEYRAVTELGRPDPE	59
CO561.pro	GTQRIRSVDRYIYNREEYLRFDSDVGEYRAVTELGRPDPE	75
pCRC201.pro	GTQRIRSVDRYIYNREEYLRFDSDVGEYRAVTELGRPDPE	76
pCRC203.pro	GTQRIRSVDRYIYNREEYLRFDSDVGEYRAVTELGRPDPE	76
Native IAs beta 1.PRO	YYNKQYLEQTRAELDTVCRHNYEGVETHTSLRR-----	92
CO561.pro	YYNKQYLEQTRAELDTVCRHNYEGVETHTSLRRLLGGEDDI	115
pCRC201.pro	YYNKQYLEQTRAELDTVCRHNYEGVETHTSLRRLLGGEDDI	116
pCRC203.pro	YYNKQYLEQTRAELDTVCRHNYEGVETHTSLRRLLGGEDDI	116
Native IAs beta 1.PRO	-----EADHVGVGVTTVYQSPGDIGQYTHEFDGDEWFYVDLDKKE	92
CO561.pro	-----EADHVGVGVTTVYQSPGDIGQYTHEFDGDEWFYVDLDKKE	155
pCRC201.pro	-----EADHVGVGVTTVYQSPGDIGQYTHEFDGDEWFYVDLDKKE	156
pCRC203.pro	-----EADHVGVGVTTVYQSPGDIGQYTHEFDGDEWFYVDLDKKE	156

FIG. 12.

Native IAs beta 1.PRO	-----	92
CO561.pro	TIWMLPEFGQLTSFDPQGGLQNIATGKYTLGILTKRSNST	195
pCRC201.pro	TIWMLPEFGQLTSFDPQGGLQNIATGKYTLGILTKRSNST	196
pCRC203.pro	TIWMLPEFGQLTSFDPQGGLQNIATGKYTLGILTKRSNST	196
Native IAs beta 1.PRO	-----L	93
		200
pCRC201.pro	PATN.	201
pCRC203.pro	PATNHHHHHH.	207

**FIG. 12.** (CONTINUED)

## INTERNATIONAL SEARCH REPORT

International application No.

PC1/0301/09616

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : A61K 35/28

US CL : 514/8, 903; 530/395, 402, 403; 424/88

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/8, 903; 530/395, 402, 403; 424/88

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAPLUS EMBASE BIOSIS MEDLINE WEST

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,194,425 A (SHARMA et al.) 16 March 1993, entire patent	1-42
Y	US 5,284,935 A (CLARK et al.) 08 February 1994, entire patent	1-42
Y	US 6,015,884 A (SCHNECK et al.) 18 January 2000, entire patent	1-20

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
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"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

16 JULY 2001

Date of mailing of the international search report

05 SEP 2001

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